The Impact of Obesity on Proteins Involved in Drug Disposition

by

Ali Mohamed Ali Abdussalam

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Abstract

The prevalence of obesity worldwide has increased in the last few decades. The risk of obesity comes from its association with various clinical diseases especially cardiovascular diseases and diabetes. In this thesis, we assessed the expression of some drug metabolizing enzymes and transport proteins in livers and kidneys of rats fed normal rat chow with water or one of three types of high calorie-containing diets.

Four groups of rats (10/group) were housed for 14 weeks and they had access either to normal rodent food and water (control), normal rodent chow and high fructose-corn syrup water (HFCS), 45% high fat (FD) diet and water, or 45% FD and HFCS. After 14 weeks, all biochemical and hematological parameters were assayed using standard diagnostic kits. The mRNA levels and protein contents of cytochrome P450 (CYP) enzymes and membrane transporters in rat liver and kidney were determined using real-time PCR and Western blot respectively. There were significant increases in plasma insulin, leptin, triglyceride and cholesterol in each of the high calorie fed groups compared to control group. Relative to the control, the mRNA and/or protein levels of CYP3A1, 3A2, and 2C11 were decreased in the liver of both FD and HFCS/FD groups. Although mean hepatic mRNA of OCT1, MATE1 did not change significantly, the transporters protein levels of OCT1 showed significant decrease in HFCS/FD and for MATE1 the decrease were significant in both FD and HFCS/FD. In renal, there were no significant differences in mRNA levels of Oct1, Oct2, Mate1, and Mdr1 between groups. In contrast, the protein levels were significantly down regulated for OCT1 in HFCS/FD, and for OCT2 in both FD and HFCS/FD.

In separate experiment Amiodarone.HCl (AM) biodistribution in presence and absence of

dietary induced obesity was studied. To achieve this, two groups have been used. The control group was fed on standard rat chow with normal drinking water and the HFCS/FD group was on high fat diet (45% kcal fat) with 13%w/v HFCS. After 14 weeks, rats in the both groups were given AM 25 mg/kg orally and euthanized by cardiac puncture at different time intervals according to the study protocol. In this single dose oral study, obesity significantly increased plasma concentrations of AM. In HFCS/FD group Liver concentration of AM was higher, however, both lung and heart concentrations was lower compared to control. Desethylamiodarone (DEA) concentrations, a main product of AM metabolism, were lower in plasma, lung and heart but higher in the liver of HFCS/FD compared to control.

Our findings suggest that obesity induced by high caloric diet can reduce the expression of some microsomal enzymes and transporter proteins. Hyperlipidemia seems to play a role in this mechanism and that the degree of its effect is enzyme and transporters dependent. These findings could explain some of changes found in biodistribution of AM in obese rats.

Preface

This thesis is an original work by Ali Mohamed Ali Abdussalam. The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, Project Name "Investigating the impact of obesity on proteins involved in drug disposition", No. AUP00000825, March 2013.

This work is dedicated to

My great parents who always been here by my side with their love, supports, and inspiration.

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List of abbreviations and symbols

°C	Degree Celsius
ABC	Adenosine triphosphate binding cassette
AgRP	Agouti related protein
ALT	Alanine aminotransferase
AM	Amiodarone
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
AUC	Area under the curve
BCRP	Breast cancer resistance protein
BMI	Body mass index
BSA	Body surface area
CART	Cocaine– and amphetamine–regulated transcript
Cl	Clearance
CM	Chylomicrons
Cmax	Maximum plasma drug concentration
CMS	Cardiac metabolic syndrome
CNS	Central nervous system
CRP	C-reactive protein
Css	Steady state concentration
CYPs	Cytochrome P450
DEA	Desethylamiodarone
DEPC	Diethyl bicarbonate
EDTA	Ethylenediaminetetraacetic acid
FD	Group provided high fat diet 45% kcal of fat and normal water
FFA	Free fatty acids
fu	Unbound fraction
GFR	Glomerular filtration rate
GIP	Gastric inhibitory polypeptide
GLP-1	Glucagon-like peptide-1 (active)
h	Hour
H_2SO_4	Sulfuric acid
HCl	Hydrochloric acid
HDL	High-density lipoprotein
HFCS	High fructose corn syrup(Group or liquid used)
HFCS/FD	Group provided high fat diet (45% kcal fat) with 13%w/v HFCS
HL	Hyperlipidemia
HPLC	High performance liquid chromatography
IBW	Ideal bodyweight
IL	Interleukin
ip	Intraperitoneal
IS	Internal standard
iv	Intravenous
Kcal	Kilocalories
KCL	Potassium chloride
Kg	Kilogram
KH_2PO_4	Potassium dihydrogen phosphate
Kp	The tissue-to-plasma concentration ratios
L	Liter
LBW	Lean body weight

LDL	Low-density lipoprotein
LP	Lipoprotein
LPDP	Lipoprotein deficient plasma proteins
LPS	Lipopolysaccharide
m^2	Square meters
MATE	Multidrug and toxin extrusion
MC	Melanocortin
MCP-1	Monocyte chemoattractant protein 1
MDR1	Multidrug resistant gene 1
mg	Milligram
min	Minutes
mL	Milli liter
mM	Milli molar
mRNA	Messenger RNA
MRP	Multidrug resistance protein
Na_2CO_3	Sodium carbonate
NAFLD	Non-alcoholic fatty liver disease
ng	Nano gram
NPY	Neuropeptide Y
OATPs	Organic anion transporting polypeptides
OCTs	Organic cation transporters
PAI-1	Plasminogen activator inhibitor
Pgp	Permeability glycoprotein
POMC	Proopiomelanocortin
PYY	Pancreatic peptide YY
RIPA	Radioimmunoprecipitation assay
rpm	Round per minute
RYGB	Roux-en-Y gastric bypass
SD	Standard deviation
SLC	Solute carrier
STZ	Streptozotocin
t ½	Terminal elimination phase half-life
TBW	Total bodyweight
TG	Triglycerides
Tmax	Time to reach Cmax
TNFα	Tumor necrosis factor alpha
UGTs	UDP-glucuronosyltransferases
UV	Ultra-violet
Vd	Volume of distribution
VLDL	Very low-density lipoproteins
WHO	World Health Organization
wk	Week
α	Level of significance
αMSH	α -melanocyte-stimulating hormone
µg	Microgram
µL	Microliter

CHAPTER 1: INTRODUCTION

1.1. Cardiac metabolic syndrome

Cardiac or cardio- metabolic syndrome (CMS), is also known as syndrome X, metabolic syndrome X, and insulin resistant syndrome (1). It has been defined in different ways, based on many clinical observations associated with the syndrome (2, 3). For example, the World Health Organization (WHO) has defined CMS based on insulin resistance as the major underlying risk factor. Therefore, evidence of insulin resistance was a prerequisite for its diagnosis. However, according to American Heart Association/National Heart, Lung, and Blood Institute, CMS is defined by having three or more of the following clinical criteria: increased waist circumference, elevated fasting glucose, elevated blood pressure, high serum triglycerides (TG) and low levels of high-density lipoprotein cholesterol (HDL) (3). Over the past decade, a global increase in the number of people with the CMS has taken place. It has been reported that about 35% of the adult population in the USA, and about 25% of the adult population in Europe have CMS (3). CMS has been associated with high liability for developing type II diabetes and increased risk of cardiovascular diseases by 61% (4, 5). Further, it has been associated with vascular diseases, including stroke, carotid artery disease, coronary heart disease, chronic kidney disease and atherosclerotic renal artery stenosis (6).

Abdominal obesity and insulin resistance are considered the main underlying causes of the syndrome. The expanded adiposity in CMS-afflicted individuals leads to an increase in release of free fatty acids (FFA) (1). FFA cause increases in production of glucose, triglyceride (TG) and very low-density lipoproteins (VLDL). Further, they can precipitate a decrease in insulin sensitivity in muscle by inhibiting insulin-mediated glucose uptake (7). The higher level of glucose leads to an increase in pancreatic insulin production

resulting in hyperinsulinemia. High insulin level is associated with both increased TG synthesis and inhibition of apoB secretion (8).

Further, hypertension in CMS has been linked to the effect of insulin on sodium reabsorption and sympathetic nervous system activation as well as to the increase in the production levels of angiotensinogen, resistin, and leptin from adipose tissue (1).

In addition CMS is linked to inflammation. The increased levels of cytokines including interleukin 6, tumor necrosis factor alpha (TNFα) and C reactive protein is associated with increased adiposity in CMS which can results in more insulin resistance, lipolysis and hepatic production of glucose and VLDL (3).

1.2. Obesity

The incidence of obesity has increased worldwide and become a major public health concern. In 2014, the World Health Organization estimated that about 39% of adult populations were overweight and 13% were obese (9). Obesity can be viewed as a chronic metabolic disease in which there is an excessive accumulation of fat in body. This abnormal fat accumulation results mainly from a discordance between energy input and output (10).

Obesity caused by multiple factors. The main factors include increase in daily energy input representative in consumption of high caloric diet rich in fat (saturated and trans fat) and carbohydrates and decrease in energy expenditure representative in insufficient physical activity. Other contributing factors include medication such as psychotropic drugs, diabetic therapy, steroid hormones and contraceptives, and other causes related to genetics, environment and psychological disturbances (10).

The excess fat in the obese body can predispose people to higher risk of various metabolic diseases particularly cardiovascular diseases, insulin resistance, type 2 diabetes, atherosclerosis and hyperlipidemia (11). It is reported that severe obesity could lead to increase the risk of type2 diabetes by 13 to 18 fold (12). Further, obesity is strongly associated with non-alcoholic fatty liver disease (NAFLD), which is one of the main causes for chronic liver disease (13).

In addition, obesity is considered as one of the top leading risk factors for worldwide death. According to Flegal KM *et al.* obesity is associated with higher mortality compared to normal weight category (14, 15). Further, studies showed that obesity could contribute to increase in cancer risk, being associated with many different cancers including esophageal, hepatic, pancreatic, colorectal, and ovarian. Also, it has been reported that body mass index (BMI) has strong correlation with decrease in survival of patients with some cancers including colon, liver, gallbladder, pancreas, and kidney (16, 17).

There are two types of adipose tissue: brown adipose tissue and white adipose tissue. Brown adipose tissue contains small fat cells with abundant vascular supply (densely innervated by sympathetic nervous system) and mitochondria (18). The ratio of brown adipose tissue depots to white adipose tissue depots is 1:200, which can be expanded in extreme cold condition. This tissue has important role in heat production (thermogenesis). This involves mitochondrial-uncoupling proteins that uncouple oxidative phosphorylation leading to produce more heat and less ATP. (18, 19)

The white adipose tissue consists of adipocytes that are monovacuolar cells contain large fat droplet (triglyceride and cholesterol ester) surrounded by thin layer of cytoplasm with

peripheral nucleus. In case of increase in energy intake the adipocytes has the ability to expand many times to be able to store the excess energy in form of triacylglycerol (TAG). However, during starving condition or increased energy demand, lipolysis of adipocytes contents occur which is mediated by hormone-sensitive lipase and monoacylglycerol lipase. These enzymes catalyze the hydrolysis reactions that convert TAG to free fatty acids (FFA). Liver and skeletal muscle uptakes the circulating FFA via fatty acid binding protein and fatty acid translocase. FFA is used by muscle and liver for generation of high-energy nucleotide adenosine triphosphate (ATP) (18).

1.3. Animal Models of Obesity

Both heredity and lifestyle factors can be involved in initiation of obesity. The heredity factors involved mutation or absence in one or more genes leading to obesity risks. In contrast, the lifestyle factors such as overeating, poor diet and insufficient exercise can influence some biological pathways and thereby could affect one or many genes expression at the same time (20). Based on that, researchers have created numerous animal models of obesity, some of them based on mutation or manipulation in specific genes and other based on exposing the animal to some factors that promote obesity such as supplementation with a high fat diet (21).

1.3.1. Genetic mutation models

In this model, the animal is lacking for specific single gene or there is dysfunction in that gene. Examples of this model are the mutations in leptin signaling pathways in the hypothalamus, and the mutations that lie downstream of the leptin-sensing neurons in the

hypothalamus.

1.3.1.1. Mutations in leptin signaling pathways

This model include: a) Lack of leptin production model: in which a mutation in ob gene of the animal leads to inability to secret leptin and eventually obesity. An example of that is “ob/ob” mouse model. b) Insensitivity to leptin model: in which a defect in leptin receptor or leptin resistance leads to morbidly obese animals. Examples of this model are leptin receptor deficient mice “db/db” mouse, and Leptin receptor-deficient rats “fa/fa Zucker rat”. Both model share the same characteristics of obesity including hyperphagia, reduced energy expenditure, hyperglycemia, hyperinsulinemia and hypothyroidism. The difference between two models is that in leptin insensitivity model the morbid obesity is associated with high levels of leptin (21).

1.3.1.2. Deficit downstream of the brain leptin receptor

In the brain, leptin targets the arcuate hypothalamic nuclei, which has two populations of neurons. Proopiomelanocortin (POMC) expressing neurons that co-express cocaine and amphetamine regulated transcript (CART), and agouti related protein (AgRP) neurons that co-express Neuropeptide Y (NPY) (22). POMC and AgRP neurons have opposing effects on food intake (23).

POMC is a precursor of many biologically active peptides including α -melanocyte-stimulating hormone (αMSH), corticotrophin and β-endorphin. These peptides have important roles in the stress response, immune system and sexual function (24). While αMSH reduces eating and increases energy expenditure by activating melanocortin (MC)

3 and 4 receptors in the paraventricular nucleus of the hypothalamus, AgRP increases eating by acting as an antagonist at the MC4 receptor (22, 23). Researchers have targeted the genes encoded for these peptides to create different models of obesity. For example, POMC knockout mouse, POMC/AgRP knockout mice, AgRP overexpression. Also, they aimed the receptors for those hormones by knocking out MC4R or MC3R or both (21).

1.3.2. Diet Induced Obesity Models

Feeding animals on high caloric diet is one of most common models to induce obesity. This model basically is trying to mimic the human obesity by replicating the human lifestyle and the dietary habit that cause obesity in animal species. Animals usually are fed on a diet rich in fat and carbohydrate for long period of time. Under this model, many ways have been used. For examples, animals that administered diet rich in calorie such as feeding on high fructose corn syrup (HFCS), or those fed on high fat diet showed significant increases in their body weights and adiposity compared to control animal on normal caloric diet (25, 26). Also, the use of both HFCS and high fat diet was also a successful model to create obese animal model (27). It is important to know that in diet-induced obesity, the used animal models mentioned in literature showed difference in the periods that the animals been kept on the high caloric diet, and in the composition of food and/or consumed fluid. Thus, this could lead to different levels of obesity and thereby unequal biological changes.

Another model of diet-induced obesity is a selected breeding of obese animal from dietary obesity models. This results in generations that can be obese even without using high caloric diet. Also, maternal high fat feeding models results in offspring that have

increased risk of obesity in their life (21).

1.4. Management of obesity

There are three available options for the management of obesity: lifestyle changes, pharmacotherapy and bariatric surgery.

1.4.1. Lifestyle changes

A complete modification for lifestyle is usually required. This includes dietary changes by decreasing the amount of the calories consumed on daily basis, increase in the level of physical activity, and behavioral therapy through individual sessions provided by dietitians or psychologists expertise (28). Obese individuals under life style modification programs may experience weight loss in a short time frame. However, maintaining this weight loss is usually difficult and in many cases they become vulnerable to weight gain again. This can be prevented by been involved in monthly weight loss maintenance sessions (29).

1.4.2. Pharmacotherapy

In some obese patients weight management by life style modification cannot be achieved. Therefore, pharmacological approaches can be used as adjuncts to lifestyle modification in appropriate patients. Pharmacotherapy weight loss is usually indicated for obese patient with BMI $\geq$30 kg/m^2. Overweight patients with BMI $\geq$27 kg/m^2 and at higher risk of obesity associated diseases can undergo pharmacotherapy too (30). The most common

drugs used to decrease body weight can be classified under two categories: i) Short-term pharmacotherapy includes phentermine, diethylpropion, phendimetrazine, and benzphetamine. Of these mostly stimulant type drugs, phentermine is the most prescribed. All short-term mentioned drugs are acting as an appetite suppressant and their most common adverse effects are insomnia and dry mouth (30, 31). ii) Long-term pharmacotherapy which includes either single drug therapy such as orlistat, lorcaserin, and liraglutide or combination therapies such as naltrexone/bupropion and phentermine/topiramate (30, 31).

1.4.3. Bariatric Surgery

Bariatric surgery is considered the most effective treatment that provides long-term sustained weight loss for morbidly obese patients with BMI ≥40 kg/m2 and who have not responded to lifestyle modification or pharmacotherapies (32). Also, it has beneficial effects on the body lipid profile, insulin resistance, adipokines levels and hepatic inflammation (33). The last few years showed a trend towards an increase in bariatric surgery worldwide. In 2011, it was estimated that over 340,000 bariatric surgeries were performed worldwide, with about 101,645 of those surgeries been in the USA and Canada (34).

Bariatric procedures are classified as restrictive, malabsorptive or both. Restrictive procedure includes gastric banding where an inflatable silicone device is placed around the upper stomach and can be controlled to increase or decrease the degree of restriction, and sleeve gastrectomy in which large portion of the stomach is removed resulting in narrow tubular stomach. Malabsorptive procedures such as biliopancreatic diversion in

which two thirds of the distal stomach is resected followed by attachment of ilium to the proximal stomach thus bypassing the duodenum and jejunum. Restrictive and malabsorptive procedure include Roux-en-Y gastric bypass (RYGB) where the stomach is divided into two pouches a small upper pouch and a larger lower pouch then the small intestine is rearranged to be connected to both pouches (30, 35). It is reported that RYGB was the most frequent bariatric surgery worldwide with about 46.6% of the cases followed by sleeve gastrectomy and adjustable gastric banding with 27.8% and 17.8% respectively (34).

1.5. Metric used to calculate doses in obesity

In the clinic, the most concern about how obesity could affect the pharmacokinetics of certain drug has to deal with best metric to use in calculating dose in obese patients. These metrics include:

1.5.1. Direct Measures of Body Composition

In these methods the fat mass in the body is calculated from person's body weight and fat free mass. The direct measures include hydro densitometry, skinfold measurement, bioelectrical impedance analysis, and dual-energy x-ray absorptiometry. However, most physicians have no access to such metrics (36).

1.5.2. Indirect Measures of Body Composition

These measures depend on available measures such as weight, height, and sex. The indirect measures include: body mass index (BMI), ideal bodyweight (IBW), lean body weight (LBW), body surface area (BSA) and adjusted bodyweight (36).

The most frequent metric used to classify overweight and obesity is BMI. BMI is calculated by dividing the total bodyweight (TBW) in kilograms by the square of the height in meters. People are classified according to their BMI into: normal if their BMIs are between 18 and 25 kg/m^2, overweight if between 25 and 29.9 kg/m^2, obese if they have BMIs more than or equal to 30 kg/m^2 and morbidly obese if greater than 40 kg/m^2 (37).

1.6. Pharmacokinetic Parameters In Obesity

Despite the importance of above parameters in estimating the required dose for obese individuals, they do not give us a complete picture about the mechanism through which obesity could affect drug disposition.

The concentration of the drug in the body depends on different factors including drug input (the rate and extent of oral absorption), distribution (volume of distribution, Vd), and elimination of drug from the body (clearance). Vd is an important parameter for determination of the loading dose and dose intervals (as it affect half life). However, it has a rather limited use for estimating chronic dosing regimens (steady state (Css)). The Vd depends on factors like physiochemical properties of administered drug, the degree of binding to plasma protein and tissue blood flow (11).

Drug clearance (CL) is the main parameter to look at when dealing with repeated (maintenance) doses. Clearance of drug is affected by factors such as the activity of metabolizing enzymes, renal tubular reabsorption and secretion, and glomerular filtration (11, 38).

Many studies reported that obesity is associated with significant changes in pharmacokinetics and pharmacodynamics of some clinically used drugs (39). The altered drug disposition in obesity comes from pathophysiological changes that are associated with obesity such as changes in total body fat, cardiac output, hepatic blood flow, and hormonal production. Further, many drug transporter proteins are involved in the absorption, distribution and excretion of drugs. Studies have shown that obesity can change the expression of number of enzymes and transporters involved in disposition of these drugs as will be explained later.

1.6.1. Obesity and Drug Absorption

Absorption is a transfer of drug from its site of administration into a central compartment (40). Many studies on the effect of obesity on absorption explained that obesity causes uncountable changes on drug absorption. However, there is not enough data could conclude the overall effect of obesity on drug absorption.

Obesity is associated with accelerated gastric emptying, increased gastric perfusion, higher cardiac output, and changes in enterohepatic recirculation that could lead to changes in drug absorption. However, many studies reported that there are no significant differences in rate and extent of absorption of orally administered drugs such as propranolol and dexfenfluramine between obese and non-obese individuals (41, 42).

Furthermore, another study explained that the absorption after subcutaneous insulin injection in patients with non-insulin dependent diabetes mellitus did not differ between obese and non-obese (43). In contrast, the absorption of enoxaparin after subcutaneous injection was found to be slower in obese compared to non-obese but the extent of absorption was complete (44).

Another factor that might influence the absorption of drug into the systemic circulation is the drug transporter proteins. These transporters are found in some sections of the small intestine and colon of the gastrointestinal tract, and can help the influx or efflux of drugs across cell membranes and thereby affect their absorption. Sugioka *et al.* reported in his study that the absorption of nelfinavir after intraduodenal administration was significantly increased in obese rats. This increase was accompanied by increase in bile and lower expression of intestinal P-glycoprotein (an efflux transporter) (45). Further evaluations of drug absorption from different routes of administration should be carried out in obese individuals to reach a definitive conclusion regarding changes in drug absorption in this population.

1.6.2. Obesity and Drug Distribution

Distribution is a transfer of drug after absorption or systemic administration into different sites in the body from the bloodstream. The distribution of a drug into specific tissue or organ depends on many factors including chemical structure of drug, its physicochemical properties, cardiac output, regional blood flow, capillary permeability, receptor mediated drug uptake and drug-protein binding (40).

Volume of distribution (Vd) of a drug is a parameter that provides an estimate of the

extent to which a drug distributes into extravascular tissues. Studies showed that Vds of some drugs are influenced by obesity. Vd is usually expressed as the absolute Vd (uncorrected for weight) or normalized to body weight such as the Vd/ TBW or Vd/ IBW. A lipophilic drug, which has a high partition (octanol/ water) coefficient, usually possesses a higher ability to penetrate into adipose tissue compare to hydrophilic drugs. Therefore, with obese individuals having an increased amount of adipose tissue, it is expected that lipophilic drug will have larger Vd in obese compared to non-obese. For example, Sufentanil (has high lipid solubility) volume of distribution has been found to increase in obesity (46). Also, Chang *et al.* reported that the Vd was significantly larger for both piperacillin and tazobactam in obese compare to non-obese individuals (47).

Moreover, changing in serum chemistry in obesity can have large effects on drug protein binding. It is reported that alterations in the serum chemistry of morbidly obese patients leads to an increase in serum concentration of alpha 1 acid glycoprotein. This increase have big effect on the unbound fraction of basic drugs in plasma and thereby affect their tissue distribution (48).

It is known that obesity often occurs alongside hyperlipidemia (HL). HL has found to influence the extent of drug absorption through increase in the lipoproteins (LP) in plasma. This has big effect especially on lipophilic drugs. Increase in plasma LP leads to increase in amount of lipophilic drug that bind to it and decrease in its unbound fraction (fu), which consequently lead to decrease in its Vd. The Vd of AM and Cyclosporine A found to decrease in rat model of HL (49, 50).

Albumin and α1acid glycoprotein are plasma proteins that bind wide range of acidic and

basic drugs respectively. These proteins are lipoprotein deficient plasma proteins (LPDP). Some drugs have tendency to bind more to plasma lipoproteins than to LPDP. Excellent examples of this are AM and cyclosporine A that found to shift in their binding from the LPDP fraction to the LP fraction especially VLDL, LDL and HDL in HL rat model (51). Overall, obesity can affect the distribution of different drugs by altering their Vds. The degree of alteration depends on: physicochemical properties of particular drug especially lipophilicity, amount of fat in the body and degree of drug protein binding, and type of protein to which drug can bind.

1.6.3. Obesity and Metabolism

Metabolism is a biochemical modification of a chemical (exogenous or endogenous substances) from one form into another. This process involves enzymes that facilitate the conversion of lipophilic compounds into highly polar metabolites, which are in most cases less active and can be excreted easily from the body. Metabolism can occur throughout the entire body, however the liver is main organ responsible for metabolism. Metabolism is divided into phase I and phase II and each phase involves different enzyme systems (40).

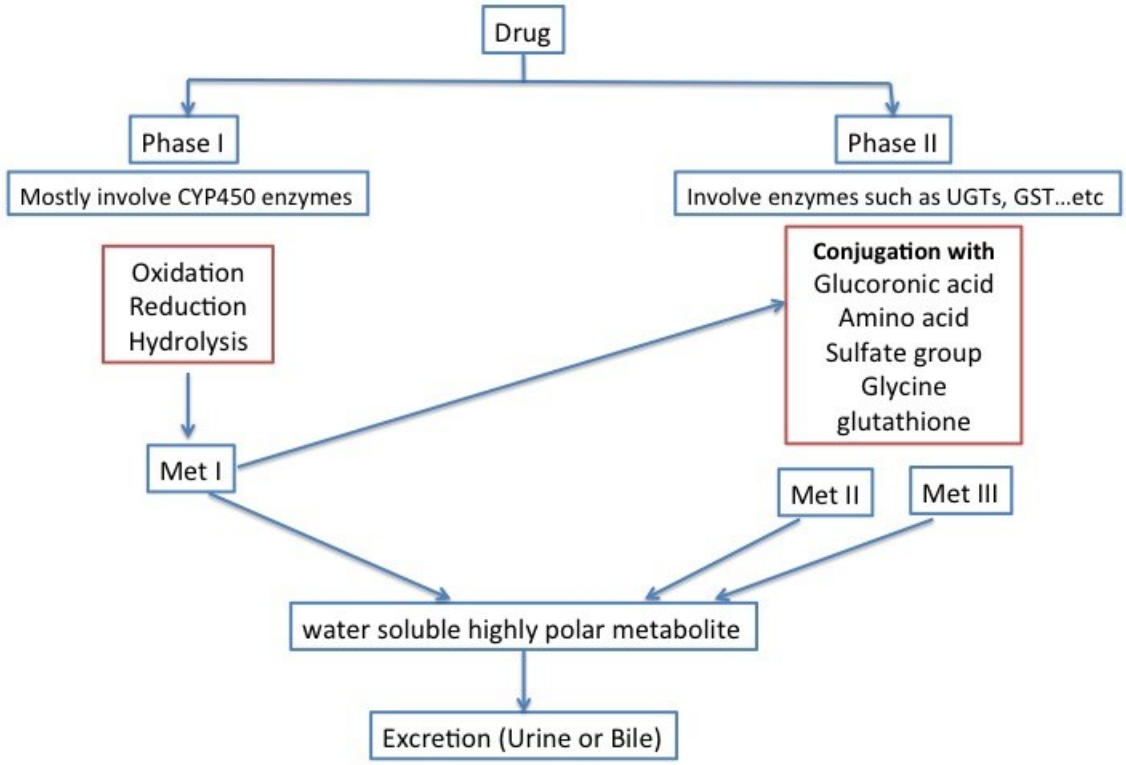


Figure1.1 Chemical reactions and enzymes involved in phase I and phase II metabolic reactions. Met = metabolite.

1.6.3.1. Phase I metabolism (mostly by CYP isoforms)

Phase I metabolism is a group of chemical reactions in which xenobiotic undergoes modification by inserting or unmasking a polar function group. Phase I reactions include mainly: oxidation, reduction and hydrolysis. The majority of phase I reactions are carried out by enzymes consist of cytochrome P450 (CYPs) (40).

CYPs enzymes belong to a superfamily of heme-containing enzymes that are responsible for oxidative metabolism of various compounds. These enzymes are membrane-bound proteins, found in the smooth endoplasmic reticulum of liver and other tissues including

brain, heart, lung, and kidney (52). There are many CYP enzymes, which have been classified into several families and subfamilies according to their amino acid sequence similarities. The most important families for drug metabolism in the human are CYP1, CYP2, and CYP3 families (53).

Many studies have shown that obesity can change the expression of a number of these enzymes. However, those previous findings have been somewhat inconsistent with regard to obesity-related effects on enzymes expression.

1.6.3.1.1. CYP3A

Cytochrome P450 3A subfamily is the most abundant enzymes in liver and responsible for about 50% of metabolism in the liver and other tissues (54, 55). CYP3A4 has reported to be down regulated in obesity. A study on guinea pig models of diet-induced metabolic syndrome demonstrated that CYP3A4 expression was decreased in the obese group (56). The protein expression of CYP3A11 (CYP3A4 in human) found to be decreased in male of genetically obese mice (ob/ob mouse), however, the females of the same strain showed decrease in mRNA expression without difference in protein levels (57). Also, in humans it was found that an increase in BMI is associated with decrease in expression of CYP3A4 in both liver and intestine (58). In contrast, Ghonium *et al.* reported that obesity induced by high fat diet in female rats showed increase in CYP3A2 expression (59).

Additionally, the pharmacokinetics of various CYP3A4 substrates have been evaluated in animal models and in human. For example, Sugioka *et al.* revealed that the area under the curve (AUC) of nelfinavir was increased as a result of decrease in liver expression of CYP3A2 in Wistar rats (45). Furthermore, triazolam and alprazolam clearance were

decreased in obese persons suggesting a decrease in the activity of CYP3A4 (60).

1.6.3.1.2. CYP2E

Cytochrome P450 2E1 is responsible for 5% of metabolism in the liver (40). The mRNA and protein expression of CYP2E1 in obese male mice found to be decreased compare to wild type (57). Yoshinari *et al.* explained that CYP2E1 was decreased in mice model of obesity induced by hypothalamic lesions (61). In contrast, another study revealed that the expression of CYP2E1 in liver and fat were increased significantly in both zucker rat fed on high fat diet and genetically obese rats compared to that of control (62). With regard to CYP2E1 activity, it was reported that CYP2E1 metabolism increased by a mean of 40% in obese individuals when compared to controls using chlorzoxazone as substrate (63). In support of the previous mentioned study, other studies on CYP2E1 substrates such as enflurane, sevoflurane and halothane have demonstrated that metabolism of these volatile anesthetics are increased suggesting an increase in CYP2E1 activity (64-66).

1.6.3.1.3. CYP2C, CYP1A

CYP2C and CYP1A account for 16% and 11% of hepatic drug metabolism, respectively (54). A few studies have reported the influence of obesity on activity of these enzymes. They found that obesity is associated with a decrease in the expression of CYP2C in mouse obesity model induced by high fat diet (61). Also, CYP1A activity found to decrease in obese mice (67).

1.6.3.2. Phase II metabolism

The phase II enzymes include various families of conjugating enzymes, such as the glutathione-S-transferases, (UDP-glucuronosyltransferases (UGTs), sulfotransferases, N-acetyltransferases, and methyltransferases. These enzymes facilitate conjugation reactions between a functional group on the xenobiotic or product of phase I metabolism and an endogenous substrate such as a glucuronic acid, an amino acid, a sulfate group, or an acetate group (40).

Acetaminophen metabolism occurs mainly by both glucuronidation and sulfation pathways, and it was found that the acetaminophen clearance was higher in obese individuals suggesting increases in activity of enzymes that mediate those pathways (68). Also, it is reported that the expression levels of UGTs 1A1, 1A6, 2B1 mRNA were lower (30–50%) in the liver of obese Zucker rats compared with lean Sprague-Dawley rats (69). In another study, they demonstrated that glucuronidation was enhanced in genetic obese Zucker rats; however, no alteration in sulfation or glutathione conjugation was seen (70).

To summarize, the previous studies showed that the expression of metabolizing enzymes can be altered by obesity. These findings showed some sort of discrepancies in change of expression of these enzymes. Different reasons might be behind these discrepancies, which have not been clarified. The change in enzymes expression could affect the metabolism of various endogenous and exogenous compounds. In regard to used medications, if enzymes levels increases, this will decrease the therapeutic effect of drug and increase its clearance. In contrast, if the enzymes level decreases, the plasma concentration of drug will increase and consequently this might lead to toxic effect.

1.6.4. Obesity and Renal excretion

Renal excretion occurs mainly through three processes: glomerular filtration, passive tubular reabsorption and active tubular secretion (40). Obesity has found to influence the renal clearance of different drugs through some of these processes. According to Henegar *et al.* the renal function was altered in obese dogs as results of increase in kidney weight, renal blood flow and glomerular filtration rate (GFR) (71). In addition, it is reported that GFR was higher in obese versus non-obese subjects. However, this difference was normalized after been calculated to lean body weight (72). In terms of tubular secretion, it reported that procainamide and ciprofloxacin tubular secretions were increased in obese individual (73). Also, ciprofloxacin clearance (in part by tubular secretion) found to be higher in obese persons (74).

1.7. Obesity and Transporter Proteins

Transporters are proteins embedded in plasma membrane of the cells and play important roles in drug absorption, distribution, metabolism and excretion. They are two types: uptake proteins that facilitate the transfer of drug from extracellular into intracellular space, and efflux proteins that pump drugs out side cells (75). Changes have been noted in both humans and animals for various transporter proteins in conditions of obesity.

1.7.1. SLC family

Solute carrier (SLC) transporters a superfamily that include 395 membrane-bound proteins classified under 52 subfamilies. This family facilitate the transport of wide variety of substrates of different structure across biological membrane (76).

1.7.1.1. OCTs

Organic cation transporters (OCTs) are members of solute carrier 22 A family (SLC22A). They are expressed in many tissues in the body. OCTs are membrane proteins that are responsible for uptake of most organic cation xenobiotics. Many clinically used drugs are substrates for OCTs. For examples, metformin (antidiabetic agent) and lamivudine (antiviral agent) Ciprofloxacin (Antimicrobial), Quinidine (Antiarrhythmic), Imatinib (Antineoplastic) (75, 77).

1.7.1.1.1. OCT1

OCT1 is a first member of SLC22A family (SLC22A1). OCT1 is expressed in many tissues, mainly at sinusoidal membrane of hepatocytes in liver, apical membrane of epithelial cells in the proximal and distal tubules of the nephron in kidneys and basolateral membrane of enterocytes in the intestine (77). Because of its location in sinusoidal membrane of hepatocytes, OCT1 plays an important role in drug metabolism of many cationic drugs by facilitating their uptake from blood stream into liver and thereby start their metabolism by CYPs enzymes (75). Obesity is observed to associate with alteration in OCT1 expression. It is reported that OCT1 expression in liver was increased in mice fed on high fat diet (78). In the same study, they found that the hepatic uptake of metformin (OCT1 substrate) was increased in obese group. Further, Moreno-Navarrete *et al.* has revealed that OCT1 expression was increased in adipose tissue of obese subjects (79).

1.7.1.1.2. OCT2

Expressed mainly in the kidneys specifically to the basolateral membrane of renal tubules. Also, it is found in lung, intestine and inner ear. It has important function in secretion of organic cation drugs in the kidney by facilitating their uptake across basolateral membrane (75). Reduction in OCT2 expression has been demonstrated in high fat diet and streptozotocin induced diabetic type II rat models (80).

1.7.1.2. MATE

Multidrug and toxin extrusion (MATE) proteins belong to the family of solute carriers (SLC47). MATE1, MATE2, MATE2K are members of this class, and they function as efflux transporters pumping their substrates out of cells (81). In human MATE1 is expressed in many organs including liver, kidney, skeletal muscle, adrenal gland, and testis (75). MATE2K has been identified in luminal membrane of proximal tubular epithelial cells of the kidney (82). MATEs are involved in transportation of many drugs including metformin, cimetidine, cephalexin, captopril and other drugs (81). Not enough information is available about the direct effect of obesity on MATE expression.

1.7.1.3. OATPs

Organic anion transporting polypeptides belongs to SLCO transporter family. They are uptake transporter proteins that play an important role in drug pharmacokinetics. They mediate the uptake of various clinical drugs such as atorvastatin (lipid lowering drug), methotrexate (antineoplastic antimetabolites), and enalapril (angiotensin converting enzyme inhibitor used in treatment of hypertension). OATPs expressed in cell membrane

of various tissues such as liver, kidneys, brain and intestine (75). With regard of its expression in obesity, OATP1A4 has found to increase in liver of obese female rats fed on high fat diet (59). Cheng *et al.* reported that the protein expression of OATP1A4 found to increase in both male and female obese mice model compare to wild type. However, the mRNA of *Oatp1a4* found to decrease in liver of these mice. Same study also revealed that the mRNA of *Oatp* transporters *1b2, 1a6, 2b1* were decreased in both sex of obese mice, however, the protein expression were difference depending on type of transporter, sex of mice and the analyzed tissue (57). Furthermore, the mRNA of *Oatp2* found to be lower in obese Zucker rats (69).

1.7.2. ABC family

The adenosine triphosphate binding cassette (ABC) transporter superfamily is the largest family of transmembrane proteins. These proteins bind ATP and use the energy to mediate transport of different compounds. This family include 48 transporters classified under seven subfamilies from A to G based on sequence homology (83).

1.7.2.1. P-glycoprotein

Permeability glycoprotein (Pgp) is an efflux transporter that belongs to ABC family transporters. It is encoded by multidrug resistant gene 1 (MDR1) and also known as ABCB1 (84). It mediates the transport of drugs from intra- to extracellular space. It is expressed in many tissues mainly in intestine, kidneys, liver, placenta, lymphocyte and blood brain barrier. The main functions are: working as defensive barrier and active excretion in the intestine, active secretion in the kidneys, mediate drugs transfer in liver,

and protecting central nervous system (CNS) against xenobiotic penetration through blood brain barrier (75). Many therapeutic drugs are substrates for MDR1 including indinavir and saquinavir (HIV protease inhibitors), morphine (analgesics), digoxin (cardiac glycosides), and erythromycin and tetracycline (antibiotics) (84).

Many studies reported that the expression of MDR1 is changed by obesity. In a study that used obesity model induced by high fat diet in female rats, they found that the hepatic expression of MDR1 was increased in obese versus non obese rats (59). In addition, Nawa *et al*. reported that the intestinal expression of MDR1 increased in hyperglycemia/hyperinsulinemia associated monosodium glutamate induced obese mouse model (85). In contrast, in the same above-mentioned study by Sugioka and his colleagues they revealed that liver and intestinal expression of MDR1 decreased by around 70 % compared to control group in obesity model induced by high fat diet in Wistar male rats (45).

1.7.2.2 MRPs

Multidrug resistance protein (MRP) subfamily is a group of efflux transporters that belong to the ABC transporters family. They are localized to the plasma membrane of different cell types and distribute in different tissues such as liver, kidney, brain, muscles, pancreas, intestine and macrophages. MRPs are efflux pumps for many endogenous compounds and xenobiotic anions, and they mediate transportation of different glucoronide and glutathione conjugates (86). Also, they play an important role in drug metabolism by mediating the last step of drug removal from the body (75). Examples of drugs transported by MRPs are adefovir and tenofovir (antivirals), ceftizoxime and

cefazolin (antibiotics), hydrochlorothiazide and furosemide (diuretics used in treatment of hypertension) (86). Many observations have been reported on the influence of obesity on MRPs transporters. Cheng *et al.* demonstrated in his work on obese mice model that the mRNA and protein expression of MRP4, MRP3 and MRP2 were increased in livers of obese mice. However, in the kidney the results showed some variation with mRNA levels being increased for *Mrp4* and decreased for *Mrp3* (57). Further, it is reported that the mRNA expression of *Mrp3* in obese Sprague Dawley (59) and *Mrp2* levels in obese Zucker rats (69) were decreased in liver of both strains.

To summarize, obesity can affect the expression of transporters in the body. The obesity related effect might vary between different transporters depending on the family that a transporter related to and also on the tissue type. In general, the changes in transporter proteins mediated by obesity are of great significance for the processes involved in drug pharmacokinetics. For example, metformin pharmacokinetics found to change with obesity. A study on the effect of gastric bypass surgery on the absorption of metformin in morbidly obese patients revealed that there were significant increases in the estimated bioavailability and renal clearance (87, 88). The combination of these two factors led to non-significant changes in plasma AUC of metformin. Metformin is a biguanide antidiabetic agent and drug of choice in treatment of diabetes type II in obese patients (89). In Padwal et al. study (88), patients' body weights were decreased by almost 30% at the time of study (after surgery). There were significant reductions in plasma levels of cholesterol triglyceride and LDL and increases in HDL plasma levels. Given that metformin is excreted mostly unchanged in the urine, it is expected that transporters are

the main reason for such changes in metformin pharmacokinetics. Metformin is a known substrate for OCT1, OCT2 and MATE (90). OCT1 is important in uptake of metformin by enterocytes into the blood stream. Also, it facilitates the uptake of metformin into the hepatocytes. MATE and OCT2 play important role in uptake of drug from blood into tubular epithelial cells and then secretion into urine. Thus, in obesity it is expected that the level of these transporters would decrease leading to decrease in absorption and thereby bioavailability of metformin, and also lead to decrease in its renal clearance. In contrast, gastric bypass surgery causes increases in transporter levels.

1.8. Obesity and Nuclear receptors

Nuclear receptors (NRs) are members of a superfamily of structurally related ligand-inducible transcription factors, which can be activated by different endogenous molecules as well as xenobiotics. These receptors include steroid receptors, thyroid receptors retinoid receptors, vitamin D receptors, estrogen receptors, and orphan receptors for which no ligand has been yet identified (91).

Pregnane X receptor (PXR, NR1I2) belongs to nuclear receptor subfamily 1, group I. PXR is highly expressed in the liver and small intestine (92). It also expressed in kidney, colon, prostate, brain, breast, heart, bone marrow, ovary, and placenta (92). PXR can be activated by xenobiotics and endobiotics including steroid hormones, St. John's wort, rifampicin, clotrimazol, phenobarbital or tamoxifen (93). PXR activation can lead to significant effect on drug pharmacokinetics, which is mediated by enhanced expression of CYPs such as CYP3A and CYP3B or some drug efflux transporters such as MRP1 (93, 94). Recently they found that in PXR-/- mice there were 29% decrease in body

weight compare to wild type mice after 12 weeks of high fat diet feeding. In contrast, PXR activation has reported to induce hypertriglycemia, fatty liver and impaired glucose tolerance (95).

The constitutive androstane receptor (CAR, NR1I3) is a third member in nuclear receptor subfamily 1, group I. CAR is expressed in the liver, lung, kidney, brain and heart. CAR is vey important in homeostasis of hormones and their precursors (95). CAR can be activated by different xenobiotics and endobiotics and thereby control the expression of a many target proteins (94). For example, the activation of CYP2B by phenobarbital is reported to be mediated by CAR, which regulate the Phenobarbital responsive enhancer module (PBREM) activation (95). Also, another study reported that CAR activation in wild-type mice increases hepatic expression of several genes involved in bilirubin-clearance such as UGT1A1, MRP2 compared to CAR-null ice (96). Further, CAR activation has reported to decrease the adiposity induced by high fat diet feeding in C57BL/6J mice. This decrease in adiposity was associated with improvement in lipid profile and insulin sensitivity (97).

The investigation of these nuclear receptors in terms of their regulation pathways and influence on enzymes/transporters expression is very important in understanding the mechanism behind changes in pharmacokinetics of drug in obesity condition.

1.9. Hyperlipidemia

HL is a metabolic disorder characterized by an excess of lipoproteins in the bloodstream. Patients with HL usually have high levels of low-density lipoprotein (LDL), TG, and

omega-6 free fatty acids (98).

HL is a one of the CMS components and is considered as one of the major risk factors for cardiovascular diseases specifically atherosclerosis and ischemic heart diseases (99, 100). Further, diabetes is sometimes accompanied by hyperlipidemia. Many studies demonstrated that diabetic patients are associated with increase in total cholesterol, TG, LDL, and decreased HDL (98). Also, HL has been correlated with obesity (101), malaria (102) and can be associated with therapeutic drugs used in medical conditions such as HIV/AIDS, cancer and mental illness (102).

There are several types of Lipoproteins (LP), all of them are synthesized in the liver except for chylomicrons (CM), which are synthesized by the enterocytes (102). LP can be classified according to their content into CM, very low-density lipoproteins (VLDL), intermediate density lipoproteins (IDL), LDL, and high-density lipoproteins (HDL) (102, 103). Pharmacokinetics of many drugs have been reported to change during HL. Changes of LP plasma concentrations in HL condition can affect drug disposition especially those bound to plasma lipoproteins. Lipophilic drugs are usually carried through the blood by plasma LP. Therefore, an increase in LP could lead to decrease in unbound fraction and thereby decrease their uptake into the cells. For drugs with moderate and low hepatic extraction ratios (E), it is expected that HL could lead to decrease in their metabolism and hepatic CL.

HL also has been shown to occur with the change in expression of some enzymes and transporters. It has been reported that HL is associated with reduction in drug metabolizing enzymes and transport proteins (104-106).

Another factor that might influence drug disposition in HL is LDL receptors. The LDL

receptor is a family of transmembrane receptors that reside on the cell surface. This family includes a number of receptors such as LDL-R and VLDL-R that share structural and functional properties and interact with a wide range if of ligands (107). LDL-R found in abundantly in the liver and responsible for regulation of plasma cholesterol mediating uptake and catabolism of plasma LDL and its main function is to mediate apoB100- and apoE-containing lipoproteins removal from plasma. In contrast, VLDL-R is found in tissue such as heart, muscle, and adipose tissue and its main function is the uptake of triglyceride-rich and apoE-containing lipoproteins (107, 108). The association of lipophilic drugs with lipoprotein in plasma could make more drugs go into liver by LDL-R receptors and thereby increase their metabolism. It is observed that the concentration of highly lipophilic drugs such as AM, halofantrin, and cyclosporine A found to increase in liver of HL subjects (104, 109, 110). Therefore, during HL it is expected that pharmacokinetics of a drug could be changed due to many factors that might counteract each other. These factors include changes in the amount of unbound fraction, changes in microsomal enzymes, and altering in cellular uptake of drug by LP receptors.

1.10. Obesity and Inflammation

1.10.1. Role of adipokines and inflammatory cytokines in obesity

The increase in white adipose tissue in obesity results in high inflammation levels which can be described as low-grade form of inflammation. Adipose tissue possesses the characteristic of an endocrine organ, and has the ability to secrete number of hormones (adipokines) including leptin and adiponectin, and inflammatory cytokines including TNFα, interleukin-6 (IL-6), MCP-1, visfatin, and plasminogen activator inhibitor (PAI-1)

(111). These secreted proteins play important roles in lipid and glucose metabolism, appetite and satiety, inflammation and immune function (18, 112).

Leptin is mainly produced in adipose tissues, but is also synthesised in other tissues including stomach, skeletal muscles and mammary epithelium. It has important role in energy homeostasis through its action in regulating appetite and the energy expenditure via its action on leptin receptors in the hypothalamus (113). Leptin has reported to be up regulated in obese individuals (114).

Adiponectin can be found in adipose tissue, cardiac and skeletal muscle. It is important in stimulation of fatty acid oxidation in the liver and muscles and stimulation of insulin secretion (115). Many studies reported that adiponectin is decreased in obesity and other diseases conditions including inflammation, heart disease, and injury. In contrast, other observations showed that increased adiponectin is associated with increased insulin sensitivity and weight loss stimulation. The proposed mechanism is through its suppression of TNFα, IL-6, and C-reactive protein and up regulation of interleukin-10 and interleukin-1 receptor antagonist (IL-1RA) (116).

TNFα is pro inflammatory cytokines that produced by adipocytes and macrophages reside in obese adipose tissue (117). It is reported that the adipocyte mRNA of TNFα and serum levels of TNFα are increased in obesity. Further, it is considered one of the underlying reasons for insulin resistant and type II diabetes associated with central obesity by its direct interfere with the signalling of insulin through its receptor (118). In contrast, using anti-TNFα therapies in obesity did not restore insulin sensitivity or glucose homeostasis back to normal (117, 119).

IL-6 is pro inflammatory cytokines released by both adipose tissue and skeletal muscle. It was found that IL-6 serum levels are increased in obesity condition but this elevation was decreased with weight loss. In addition, IL-6 high levels are responsible for the increase in C-reactive protein (acute-phase proteins) in obese individuals (19). TNFα and IL-6 together with IL-1b and IL-8 are involved in the induction of low-grade inflammation in obesity (19, 117).

In obese individuals, increased in BMI is associated with increase in filtration of macrophages. These macrophages have the ability to secrete a number of inflammatory cytokines including TNFα, (IL)-6 and C-reactive protein. Therefore, in obesity the high levels of TNF-α, IL-6, MCP-1, visfatin, and PAI-1 are contributed to their production by both macrophage and adipocytes. In contrast, Leptin and adiponectin are produced by adipocyte only (19, 112).

1.10.2. Inflammation and altered expression of metabolizing enzymes and drug transporters

The increase in adiposity as well as macrophage infiltration during obesity leads to increase in plasma cytokines levels. These cytokines have been linked to change in expression of some metabolizing enzymes and transport proteins. TNFα and (IL)-6 have been reported to inhibit the hepatic cytochrome P450 CYP3A4 and CYP2C8 (120, 121). In another study they found that when human hepatocytes exposed to different concentrations of TNFα and IL-6 there were decrease in expression of transporter proteins including OATP1B1/1B3/2B1, OCT1, and OAT2. The same research group found that IL-6 was associated with decrease in MDR1, MRP2/4, and breast cancer

resistance protein (BCRP). In contrast, no changes in expression were found with TNFα. Also, they reported that IL1-β is associated with decrease in mRNA levels of *OATP-B*, *OATP-C*, and *OATP8* and *MRP2/3/4*, and *BCRP* in human hepatocytes (122, 123).

In the study of inflammation induced by using of bacterial lipopolysaccharide (LPS), they found that the experimental animals injected with LPS demonstrate decrease in expression levels of many P450 mRNAs in the rats and mice livers. This was accompanied by increase in the level of inflammatory cytokines such as TNFα. It is reported that LPS caused decrease in expression of CYP1A2, 2A1, 2C6, 2C7, 2C11, 2C23, 2E1 and 3A2 in rats. Also, LPS has found to down regulate the mRNAs of *Cyp1a2, 2a5, 2c29, 2e1*, and *3a11* in the mice (124, 125). The underlying mechanism is that the injected LPS stimulate macrophages to release IL1-β and TNFα which in turn lead to secretion of IL-6 and chemokin from the other cells types, this leads to recruitment of neutrophils to the site of inflammation and production of nitric oxide and reactive oxygen species which can bind to and irreversibly modify biomolecules such as proteins (126).

1.11. Amiodarone

One drug that has been well studied in hyperlipidemic animals is amiodarone (AM). AM is a lipophilic benzofuran derivative. It belongs to a class of drugs called Vaughan-Williams class III antiarrhythmic drugs (40). AM is available as intravenous and oral doses. The IV drug is used in treatment and prophylaxis of acute ventricular tachycardia or fibrillation. The oral AM is very widely used drug to treat recurrent ventricular arrhythmias such as recurrent ventricular fibrillation and recurrent unstable ventricular

tachycardia that are resistant to other drugs and also in maintaining sinus rhythm in patients with atrial fibrillation (40, 127).

1.11.1. Pharmacological action of AM

AM exerts its pharmacological action through multiple mechanisms, including potassium channel blockade which lead to prolongation of the action potential duration and refractory period of cardiac tissue, calcium and sodium channel blocking activity and noncompetitive α and β adrenoreceptor antagonism. Moreover, AM decreases heart rate and has mild direct negative inotropic activity. It can also result in peripheral arterial vasodilation and coronary artery vasodilation (40, 128).

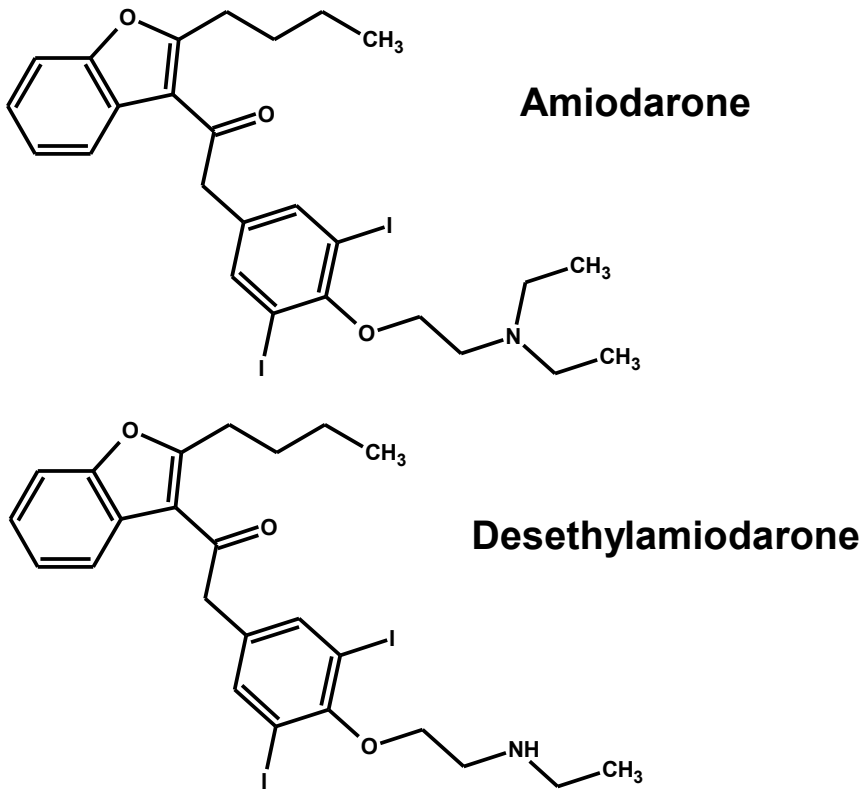


Figure 1.2. Chemical structure of amiodarone and it metabolite (Desethylamiodarone).

1.11.2. Pharmacokinetics of AM

The absorption of AM is incomplete with bioavailability ranges from 22 to 95%. AM has volume of distribution of 66 L/kg in human and extensively bound to plasma proteins especially plasma lipoproteins; it has an extended terminal phase half-life in humans of many days. Because of that very long period of time is needed before the drug reach equilibrium (128). Because of this long half-life and its very slow elimination, AM is given as one single dose per day. Further, AM has a low hepatic extraction ratio in humans but a moderate extraction ratio in rat (40, 129).

The therapeutic plasma concentration of AM range between 0.5–2 μg/mL but because of its slow tissue accumulation, high loading dose of 1200–1600 mg/day is required for several weeks before maintenance dose of 200-400 mg/day is started (130).

AM undergoes biotransformation mainly in the liver. Several cytochrome P450 (CYP) isoenzymes have been implicated in AM metabolism in human including CYP3A4, 2C8, 1A2 and 2D6 (131). There are different metabolic pathways involved in AM metabolism including N-deethylation, hydroxylation, O-dealkylation, deiodination, and glucuronidation. The products of these pathways include mono-N desethylamiodarone (DEA), di-N –desethylamiodarone and deiodinated desethylamiodarone (132, 133). The most important pathway is N-dealkylation which result in formation of DEA. DEA has pharmacological and toxicological activities similar and sometimes more potent than that of AM. It is clinically important to know plasma and tissue concentrations of DEA. Studies showed that increase in DEA/AM serum concentration ratios is associated with increase in pulmonary toxicity (134). Also, they found that DEA in vitro has more

inhibitory effect on CYP3A4 and MDR1, which results in inhibition of AM metabolism and transportation.

Many adverse effects may develop during AM therapy. The most serious side effect is pulmonary fibrosis, which can be fatal. Microdeposits, hepatic dysfunction, corneal epithelial deposits, peripheral neuropathy, proximal muscle weakness, and symptoms of thyroiditis and thyrotoxicosis might develop during chronic treatment with AM (130). Photosensitivity and hyperpigmentation are other side effects which concur with AM treatment (135).

1.12. Rationale, Hypotheses, Objectives

1.12.1. Rationale

The increase in the BMI during obesity is attributed to the imbalance in energy consumption and expenditure, which leads to fat accumulation. Obesity can be associated with many metabolic diseases including cardiovascular diseases, insulin resistance, type 2 diabetes, atherosclerosis and hyperlipidemia. Therefore, multidrug therapy in obese people is required to treat these different disorders. However, the knowledge about the disposition of those medications in obese individuals is still unclear.

Increased adiposity is reported to be associated with changes in Vd of some drugs especially those with high lipophilic characteristics. Also, clearance of some drugs has been reported to change in obesity. Changes in the distribution and clearance profile of a drug could lead to change in its other pharmacokinetics parameters and consequently leads to an alteration in pharmacological response of that drug.

Many drugs are substrates for a variety of metabolizing enzymes and transporter proteins, which have been reported to change in obesity conditions. However, the mechanism behind these changes has not been adequately studied. Obesity has characteristic of inflammation process and can be considered as chronic low-grade form of inflammation due to the increased number of infiltrated macrophages into the adipose tissue. Both adipose tissue and macrophages has the ability to secrete number of cytokines such as TNFα, which is known to influence the expression of drug transporters and metabolizing enzymes.

Moreover, obesity is usually associated with HL. HL has been reported to associate with decrease in expression of some drug metabolizing enzymes and transport proteins (104-106). Further, increasing in the plasma lipoprotein concentrations have effect on the distribution of drugs and consequently their metabolism and excretion. The high level of LP in hyperlipidemia condition can increase the binding of drugs to the LP fraction and decrease the unbound fraction especially for those drugs with high binding affinity to LP. This could lead to decrease in Vd and also affect the clearance of the drug. LP receptor can play important role in drug disposition by facilitating the uptake of LP-bound drugs into tissues such as liver. Therefore, HL could alter the drug pharmacokinetics through its effect on unbound fraction or through its effect on drug metabolizing enzymes and transport proteins.

1.12.2. Hypotheses

1. Dietary induced obesity causes decreases in drug transporters expression with consequences on drug metabolism and excretion compared to control rats.
2. Dietary induced obesity causes decreases in metabolizing enzymes expression leading to decrease in drug metabolism and increase in its plasma concentration compared to control rats.
3. Hyperlipidemia associated with obesity could be involved in the changes that we might see in hepatic metabolizing enzymes levels and hepatic and renal transporters levels.
4. Changes in the inflammatory cytokines and adipokines could be one of the reasons behind the expected changes in the protein and enzymes levels.

5. The change in metabolizing enzymes and transporters expression could affect the biodistribution of amiodarone in dietary induced obese rats compared to lean rats.

1.12.3. Objectives

1. To develop a nutritionally obese rat model that mimics human obesity.
2. To determine the expression of number of solute carrier (SLC) membrane transporters including OCT1, OCT2, and MATE1.
3. To determine the expression of member of ATP binding cassette membrane efflux transporter, MDR1.
4. To assess the effect of obesity on the expression of hepatic metabolizing enzymes (CYP3A1, CYP3A2, CYP2C11, CYP2C11 and CYP1A1)
5. Using the high performance liquid chromatography (HPLC) method to determine whether dietary induced obesity results in clinically significant changes in biodistribution characteristics of AM in obese vs. normal weight rats.

CHAPTER 2: MATERIAL AND METHODS

2.1. Materials

Amiodarone HCl (AM) and ethopropazine HCl, were purchased from Sigma-Aldrich (St. Louis, MO). Desethylamiodarone (DEA) was obtained as a gift from Wyeth Research (Monmouth Junction, NJ). Methanol, HPLC water, hexane, acetonitrile (all HPLC grade) were purchased from EM Scientific (Gibbstown, NJ). Enzyme linked immune sorbent assay kits (high sensitivity) for cholesterol, HDL, and triglyceride were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Heparin sodium injection 10,000 U/mL, was purchased from Leo Pharma, Thornhill, ON, Canada), and isoflurane USP 99.9% was purchased from Pharmaceutical Partners Of Canada Inc. (Richmond Hill, ON, Canada).

TRIzol reagent and UltraPure distilled water were purchased from Invitrogen (Carlsbad, CA). High capacity complementary DNA (cDNA) Reverse Transcription Kits, 96-well optical reaction plates, SYBR Green Super Mix, and optical adhesive films were purchased from Applied Bio systems (Foster City, CA, USA). Real time PCR primers for metabolic enzymes and transporter proteins were synthesized and purchased by Integrated DNA Technologies, Inc. (Coralville, IA, USA) based on previously published sequences in the literature.

Radio immune precipitation assay (RIPA) lysis buffer system was purchased from Santa Cruz Biotechnology (Texas, USA). Page Ruler Plus Protein Ladder was from Thermo Scientific (Grand Island, USA), Bovine serum albumin (BSA) from Fisher Scientific (Ottawa, Canada). Bromophenol blue, ammonium persulphate, β-mercaptoethanol, 40% acrylamide, N,N,N',N'-tetramethylethylenediamine (TEMED) and nitrocellulose membrane were purchased from Bio-Rad Laboratories (Hercules, CA).

CYP1A1, CYP3A1, CYP3A2, and CYP2C11 primary antibodies were purchased from

BD Biosciences (Mississauga, ON). The primary antibodies (MATE1, MDR1, OCT1, OCT2, and β-actin) and secondary antibodies (Anti-mouse, anti-goat and anti-rabbit IgG peroxidase) were purchased from Santa Cruz Biotechnology, Inc (Santa Cruz, CA). Chemiluminescence Western blotting detection reagents were from GE Healthcare Life Sciences (Piscataway, NJ).

2.2. Methods

2.2.1. Animals model of obesity

2.2.1.1. Dietary model

There were three dietary interventions used in the studies. This consisted of a high carbohydrate and high fat component, given alone or in combination, and a control normal diet group. The high caloric components were 1.) a 13% w/v high fructose corn syrup (HFCS) (prepared in our lab) and 2.) a high fat content diet consisting as rat pellets (45% kcal of fat, Harlan Laboratory, Inc.). A standard rodent diet and normal drinking water were used in addition. Detailed information about the components and their caloric content are listed in Table 2.1 and 2.2.

Table 2.1. Standard Rodent Diet versus Adjusted Calories Diet composition and the percentage of calories provided by each component.

Nutrients	Calories provided %	
	Standard Rodent Diet 3.35 Kcal/g	Adjusted Calories Diet 4.6 Kcal/g
Protein	29.8	19
Carbohydrate	56.8	36
Fat	13.4	45

Table 2.2. High fructose corn syrup composition and the percentage of calories provided by each component.

Nutrients	Calories provided % (0.466 Kcal/mL)
Fructose	55
Glucose	45
Maltose	3

2.2.1.2. Animal model

All experimental procedures involving animals were approved by the University of Alberta Health Sciences Animal Policy and Welfare Committee. A total of 72 male Sprague-Dawley rats about 4 weeks of age and weighing between 80-100g were purchased from (Charles River, CRC, QC, Canada). All rats were housed two per cage in temperature-controlled room with 12 h dark/light cycle.

Two experiments were performed. The first experiment was used to assess the affect of different high caloric diets induced obesity on plasma biochemical parameters and on the expression of metabolizing enzymes and transporter proteins in both liver and kidney tissues. In this experiment, 40 rats were divided into 4 groups (n = 10 rats per group) and each group has specific type of food and drink for 14 weeks as following: The first group (Control) has been fed on standard rat chow with normal drinking water. The second group (HFCS) was given standard rat chow and 13%w/v HFCS. The third group (FD) was provided high fat diet 45% kcal of fat and normal water, and the fourth group (HFCS/FD) received high fat diet (45% kcal fat) with 13%w/v HFCS.

Free access to food and fluids was permitted throughout the entire period. Rat weights, food and fluid consumption were measured biweekly. After 14 wk, all animals were exsanguinated by cardiac puncture under anesthesia with isoflurane after 12 h fasting. Thereafter, blood samples were collected in tubes containing anticoagulant (EDTA) and plasma separated by centrifugation at 3000 rpm for 10 min. Liver and kidneys tissues were harvested into clean tubes. All samples were kept at -80 °C until the day of experiment.

The second experiment was devised to assess the effect of dietary induced obesity on AM. A total of 32 rats were allocated into two groups of 16 rats per group and allowed free access to food and water for 14 weeks. The control group was fed on standard rat chow with normal drinking water and the HFCS/FD group was on high fat diet (45% kcal fat) with 13%w/v HFCS. After 14 weeks, rats in the both groups were given AM HCl 25 mg/kg orally and euthanized by cardiac puncture at different times postdose according to the study protocol.

2.2.2. Biochemical analysis

Fasting plasma samples were assayed from 40 rats allocated to the first experiment in this study. In those samples cholesterol, TG and HDL were determined using enzymatic colorimetric assay kits according to the manufacturer's instructions. All plasma samples were measured in duplicate and the average of the two values was taken and used for statistical data analysis.

Plasma concentrations of insulin, leptin, adiponectin, amylin (active), gastric inhibitory polypeptide (GIP), glucagon-like peptide-1 (active) (GLP-1), ghrelin, Pancreatic peptide YY (PYY), and TNFα were assayed by Eve Technology Corporation (Calgary, AB, Canada). Glucose, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) analysis were performed by the Surgical Medical Research Institute, Department of Surgery, University of Alberta.

2.2.3. Real Time PCR

2.2.3.1. RNA Isolation

Total RNA from liver and kidney tissues were extracted by TRiazol reagent according to the manufacturer's instructions. In brief, 1 mL of TRIzol reagent was used per 100 mg of tissue to homogenize frozen tissue samples. Then, 1 mL of homogenate was transferred into microcentrifuge tube and 200 µL of chloroform were added to each sample, and shaken vigorously for 15 s. Then samples were incubated at room temperature for 5-10 min. This was followed by centrifugation at 12000 x g for 15 min at 4 °C. The resultant supernatants were collected into separate microcentrifuge tubes and 500 µL of isopropyl alcohol were added for each tube followed by mixing for 10 s and then incubated at -20 °C for 2 hours. After incubation, samples were centrifuged at 12000 × g for 10 min at 4°C. The formed pellet was washed with 500 µL of 75% ethanol in diethyl bicarbonate (DEPC) treated water. Thereafter, samples were centrifuged at 12000 g and 4 °C for 10 min to precipitate the RNA. Then, 50 µL DEPC treated water were added to each sample. The samples were subjected to vortex mixing for a few seconds and incubated at 60°C in a water bath (15 min). All sample were stored at -80 °C until further analysis. The total RNA was quantified by measuring the absorbance at 260 nm. The quality of the isolated RNA was determined by measuring the 260/280 ratios.

2.2.3.2. cDNA Synthesis

The synthesis of the first strand cDNA was performed using a high-capacity cDNA reverse transcription kit according to manufacturer's instructions. In brief, 1.5 µg of total RNA from each tissue sample was added to a mixture of 2.0 µL of 10x reverse

transcriptase buffer, 0.8 µL of 25x dNTP mix (100 mM), 2.0 µL of 10x reverse transcriptase random primers, 1.0 µL of MultiScribe reverse transcriptase, and 4.2 µL of nuclease-free water. The final reaction mixture was kept at 25°C for 10 min, then heated to 37°C for 120 min. Thereafter, the samples were heated for 85 °C for 5 s, and then cooled to 4°C.

2.2.3.3. Quantification by Real-Time PCR

Quantitative analysis of specific mRNA expression was performed by real-time PCR by subjecting the resulting cDNA to PCR amplification in 96-well optical reaction plates using the ABI Prism 7500 System (Applied Biosystems, Foster City, CA, USA). The 25 µL reaction mix contained 0.1 µL of 10 µM forward primers, 0.1 µL of 10 µM reverse primers, 12.5 µL of SYBR Green Universal Master mix, and 11.05 µL of nuclease-free water and 1.25 µL of cDNA sample. All primer sequences used in this study are listed below (Table 2.2). No-template controls were incorporated to test for the contamination of any assay reagents. After sealing the plate with an optical adhesive cover, the thermo cycling conditions were initiated at 95°C for 10 min, followed by 40 PCR cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min. Melting curve (dissociation stage) was performed at the end of each cycle to confirm the specificity of the primers and the purity of the final PCR product.

2.2.3.4. Real-time PCR data analysis

The real-time PCR data were analyzed using the relative gene expression, i.e., the $2^{-\Delta(\Delta C^{T})}$ method as described in Applied Biosystems User Bulletin No. 2 and explained

further by Livak and Schmittgen (136). All data are presented as fold change in gene expression normalized to the endogenous reference gene (β-actin) and relative to the untreated control.

Table 2.3. Rat primer sequences used for real-time PCR reactions:

Gene	Forward primer	Reverse primer
Cyp3a1	ATG TTC CCT GTC ATC GAA CAG TAT G	TTC ACA GGG ACA GGT TTG CCT
Cyp3a2	GCT CTT GAT GCA TGG TTA AAG ATT TG	ATC ACA GAC CTT GCC AAC TCC TT
Cyp2c11	TGA AGG ACA TCG GCC AAT CA	TTT CTG TGC CAG CTC CAA AC
Cyp1a1	TGA GAC AGT ATT GTG TAG TCC AAG T	CAC TTG GTA GGG TGG TAA AAG C
Oct1	TCC TGC TGA CCT GAA GAT GCT	GAA CAG GTC GGC AAA CGA A
Oct2	CCA TGT CGA CCG TGG ATG ATA	TCA ATT TCT TGC CAG CGT CC
Mate1	TAT AAC GGG CAT CGC TGC TA	ATA GGC CCA CCA CTC TAT GC
Mdr1	GAC AGG ACA TCA GGA CCA TCA AT	GAC GTT TTC TCG GCC ATA GC
β-actin	GCA GGA GTA CGA TGA GTC CG	ACG CAG CTC AGT AAC AGT CC

2.2.4. WESTERN BLOTTING

2.2.4.1. Protein separation

Protein separations from liver and kidney tissues were done using previously described method (137, 138). In brief, for CYP450s: tissue homogenates in cold sucrose solution (0.25 M) were centrifuged at 12000 rpm. Thereafter, supernatants were separated from the previous step and further centrifuged at 100,000 g for 1 h. Then, the resulting pellets containing the proteins were re-suspended in cold (4°C) sucrose and quantified using the Lowry method (139). For transporters: 300 mg of tissues were homogenized using RIPA [1x Lysis Buffer: (1x TBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 0.004% sodium azide), PMSF, protease inhibitor cocktail, sodium orthovanadate] buffer on ice, then centrifugation at 18000 rpm for 15 min was applied and the resulting supernatants containing the proteins, were quantified using the Lowry method.

2.2.4.2. Lowry assay method for protein concentration in microsomal preparations

Serial standard solutions of BSA were used for the quantification of unknown protein concentrations. For the determination of protein concentrations in liver and kidney samples, Reagent A consisting of 1 mL of cupric sulfate 1% in distilled water, 1 mL of sodium and potassium tartarate 2% in distilled water, 20 mL of 10% Na_2CO_3 anhydrous in 0.5 M sodium hydroxide, and reagent B comprising of 1/10 diluted solution of Folin-Phenol reagent in distilled water were used. The BSA stock solution of 500 μg/mL was used for preparing the working standard solutions (0, 100, 200, 300, 400 and 500 μg/mL) of BSA in distilled water.

For the determination of unknown protein concentrations, 250 μL of reagent A was added

to the mixture of 2 µL of protein sample and 248 µL of distilled water (unknown concentration of protein) or 250 µL of each standard solution. This reaction mixture was incubated at room temperature for 10 min after which 750 µL of reagent B was added to each of the test tubes under continuous vortex mixing, and samples incubated at 50 °C for 10 more min. At the end of the reaction, 200 µL of each mix was transferred to the ELISA plate and analyzed using an ELISA reader at 550 nm.

2.2.4.3. Protein sample preparation

Each protein sample was denatured by diluting with 2X loading buffer and boiling. For CYP450s, 50 µg microsomal proteins were dissolved in 2X loading buffer and then boiled for 5 min at 100 C^0. For transporters, 60 µg of proteins were dissolved in 2X loading buffer and heated for 20 min at 36ºC.

2.2.4.4. Western Blot Analysis

Proteins in each denatured sample were separated by 10% SDS-polyacrylamide gel (SDS-PAGE) and electrophoretically transferred to a nitrocellulose membrane. Then, membranes were blocked overnight at 4 C^o using blocking solution contains 25 mM Tris-base (TBS), 5% skim milk, 2% bovine serum albumin, 0.15 M sodium chloride, 3 mM potassium chloride, and 0.5% Tween-20. Thereafter, the blocking solution was removed and the blots were rinsed three times in a wash buffer (0.1% Tween-20 in Tris-buffered saline). Then, the membranes were incubated with primary antibody for 2 h at room temperature. The primary antibody solution was removed and blots were washed as previously described, followed by incubation with secondary antibody for 1 h at room

temperature. Rinsing was then applied to remove the extra secondary antibody. Finally, the protein bands were detected using enhanced chemiluminescence. Protein band intensities (relative to β-actin bands intensity) were quantified using Image-J software (National Institutes of Health, Bethesda, MD, http://rsb.info.nih.gov/ij.).

2.2.5. Biodistribution Study

2.2.5.1. Animal treatment and sample collection

To assess the effect of obesity on the tissue distribution of AM and its metabolite (DEA), rats were given doses of AM. Each rat in the control and HFCS/FD groups was given an AM oral dose of 25 mg/kg and each group was further stratified into 5 time points. At 1, 3, 6, 9, or 24 h post dose, each rat (n = 3-4 rats per time point were used) was euthanized under anesthesia by cardiac puncture. At each time point, heart, liver and lung were harvested. Also, blood was collected and plasma was separated as previously described. All samples were kept in -80 °C until the day of experiment. AM and DEA levels were assessed using HPLC method explained below.

2.2.6. Assay of amiodarone and desethylamiodarone

A validated published assay for the quantitation of amiodarone and desethylamiodarone was used (140, 141). A brief description of the method follows.

2.2.6.1. Stock Solutions

Stock solutions of 0.1 mg/mL of AM, DEA and internal standard (IS, ethopropazine)

were prepared in methanol. Various working concentrations of 0.1, 1, 10 µg/mL were prepared for generation of standard curves for both AM and DEA. An IS stock solution of 0.1 mg/mL was used. All stock solutions were stored at –20 ºC.

2.2.6.2. Extraction Procedure

AM, DEA, and IS were extracted from 100 µL rat plasma using previously published liquid-liquid extraction method (140). In brief, 30 µL of IS was added to each plasma sample. Then, to extract the analytes, 300 µL of acetonitrile was added followed by vortex mixing for 5-10 s and centrifugation for 2 min in order to precipitate plasma proteins. Thereafter, the supernatants were transferred to new test tubes and 300 µL of HPLC water followed by 3 mL of hexane were added. After vortex mixing for 30 s and centrifugation at 3000g for 3 min, the organic layer was transferred into clean tubes and dried *in vacuo*. The dried residue was reconstituted in 150 µL of mobile phase and 130 µL was injected. For extraction from tissues, approximately 300 mg of each tissue was homogenized in distilled water in ratio of (1:3 w/w) with high-speed tissue homogenizer. From each tissue total homogenate, an aliquot of 400 µL (equivalent to 100 mg of wet tissue) was transferred to a clean glass tube, and then 30 µL of IS and 1 mL of acetonitrile were added. Thereafter, the tubes were vortex mixed for 10 s and centrifuged at 3000g for 3 min to precipitate the protein content. Then, the supernatant layers were transferred to new tubes and 6 mL of hexane were added. Similar to extraction from plasma, all tubes were vortex mixing for 30 s and centrifuged for 3 min, then the organic layers were transferred into new tubes and dried *in vacuo*. The dried residue was reconstituted in 150 µL of mobile phase and 130 µL was injected.

2.2.6.3. HPLC conditions

The HPLC system consisted of a Waters 717 plus auto sampler system, Waters 600 multi-solvent delivery system, a guard column and Waters 486 tunable absorbance detector. Data collection and integration were accomplished using Ezchrom software computer based integrator. Chromatographic conditions were the same as described before (141, 142). The column used in this experiment was C18 analytical column (150 mm×4.6 mm with 5μm particle size). The mobile phase consisted of methanol: acetonitrile: phosphate buffer [25 mM KH_2PO_4: 3 mM H_2SO_4: 3.6 mM triethylamine] in a combination of 63:12:25 v/v. Before using, the mobile phase was degassed by passing it through a 0.45 um filter and then pumped at an isocratic flow rate of 1 mL/min. Detection was accomplished by UV absorption at 254 and 242 nm. First, the UV detection wavelength was set at 254 nm which represents the UV maximum of IS. After 9 min post-injection, it was switched to 242 nm which represents the UV maximum of AM and DEA.

2.3. Statistical and Data analysis

All data were reported as mean±SD unless indicated. Data following a normal distribution were analyzed using a one-way ANOVA followed by (Post-hoc Tukey multiple comparison test). Non-normal data was analyzed using a Kruskal Wallis one-way ANOVA followed by Dunn's method of pairwise multiple comparison procedure. The results were considered statistically significant when $p < 0.05$. Except for biodistribution studies, all other data analysis was performed using Sigma Plot 13.

For biodistribution studies, the area under the AM and DEA concentrations vs. time curves (AUC) based on the mean data from each sampling time point were calculated for each of the tissues and for the plasma. Because of study design, the AUC could not be determined for each individual rat of both groups. Therefore, the Bailer's method was used to perform the data analysis (143). In this method, α was 0.05 and the critical value of Z (Z_{crit}) for the 2-sided test after Bonferroni adjustment was 2.24, and the observed value of Z (Z_{obs}) was calculated. The maximal concentration (Cmax) and the time to achieve it (Tmax) were determined by visual inspection of the data. The tissue-to-plasma (Kp) concentration ratios were also determined for the post-distributive phase in liver, heart, and lung samples.

CHAPTER 3: RESULTS

3.1. Body Weight, Caloric Intake and Fat Mass Differences

3.1.1. Body weight

There were significant increases in the body masses of rats fed high caloric diets compared to control rats. The FD and HFCS/FD groups showed the highest weight gains compared to the other groups (Fig. 3.1). By the third week of the study HFCS, FD, HFCS/FD groups had already gained 4.23, 40.4, 37.6% more weight than the control rats. The weights of FD and HFCS/FD rats started significantly differ from control rats in week 4 and the differences increased with time (Figure 3.1). However, significant increases in HFCS rat weights started at week 9 of the study. By the end of the study the final average body weights were 639, 719, 789, 802 g for control, HFCS, FD and HFCS/FD groups respectively.

3.1.2. Caloric intake

Food and water consumption were measured twice week for 14 weeks in all groups. Control rats consumed larger amount of food compare to other groups, but because of the higher caloric density of the high-fat chow, the caloric consumption per gram in FD and HFCS/FD groups were 36% greater than that of control rats (Fig. 3.2). Fluid consumption was higher for HFCS rats, indicating that there was a preference for the HFCS-containing water (Fig. 3.3). The amount of calories from HFCS consumed by HFCS and HFCS/FD fed-rats were equivalent to 0.466 Kcal/mL. The role of HFCS in causing decreased solid chow consumption was confirmed when HFCS was omitted from the control and FD group conditions. The order in mean area under the curve for caloric consumption over 14 weeks from highest to lowest were HFCS/FD > HFCS > FD > control at 11540,

11458, 11454, and 9590 Kcal/14 weeks/rat respectively. In general, since week 4, the caloric intakes (calories/day) of HFCS, FD and HFCS/FD groups were higher than that of control group until the end of study as shown in (Fig. 3.4)

3.1.3. Perinephric fat

The weight of fat mass around the kidneys was measured. A significant gain in perinephric fat mass were observed in all high caloric diet groups compared to the control. The FD group had the highest fat mass between all groups. The mean values were 13.5 g, 27.3 g ($p < 0.05$), 44.8 g ($p < 0.05$) and 41.5 g ($p < 0.05$) for control, HFCS, FD and HFCS/FD groups respectively.

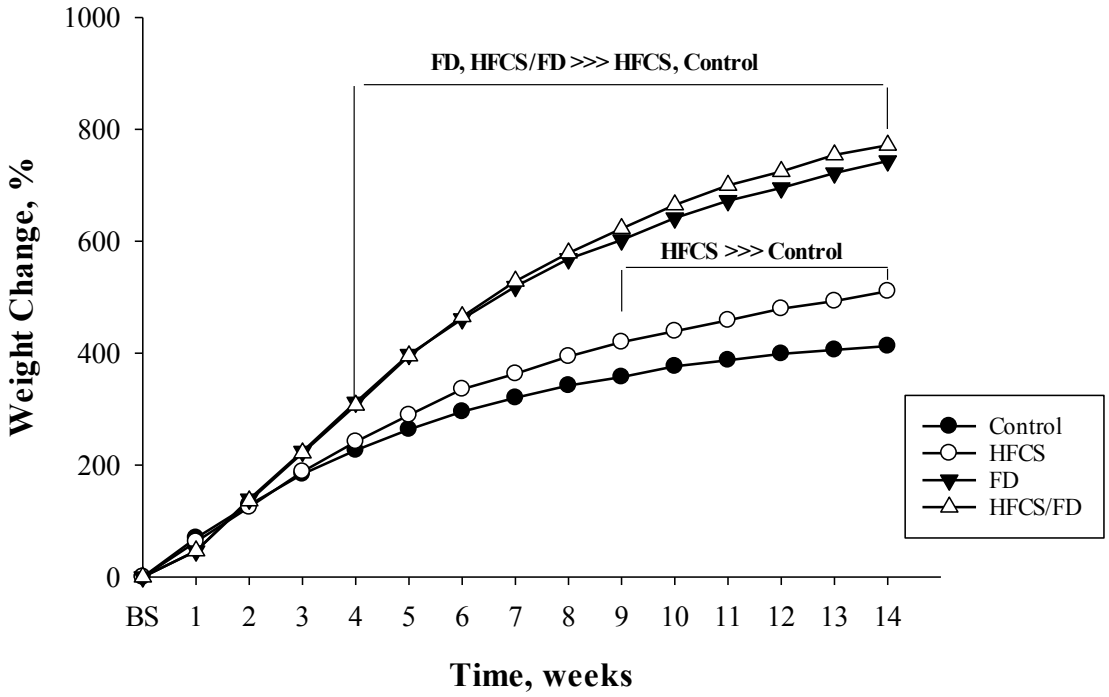


Figure 3.1: Weekly average weight gain (g) of all groups. Rats were fed on: (Control) standard diet + normal drinking water, (HFCS) standard diet + HFCS, (FD) high-fat diet + normal drinking water or (HFCS/FD) high-fat diet + HFCS for 14 weeks as described in the 'Experimental methods' section. Values are means ± SD (n 10). From 4 weeks on, the FD and HFCS/FD groups were significantly greater ($p < 0.05$) than the other 2 groups. From 9 weeks onwards, the HFCS group was significantly higher in weight gain than the control group ($p < 0.05$).

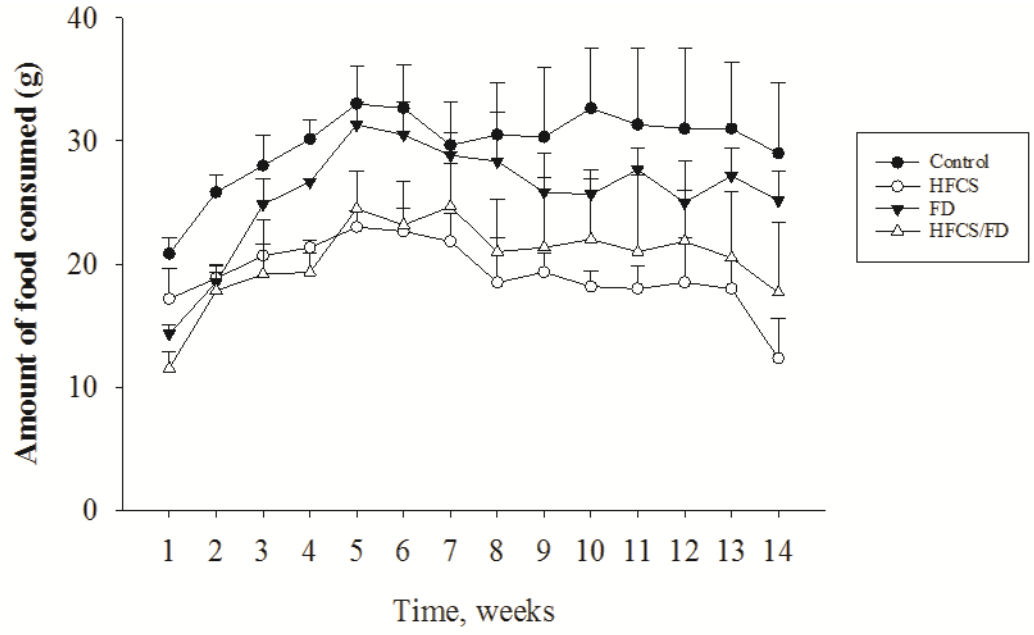


Figure 3.2: Average food consumed per rat from each group. Groups were fed either: standard diet (Control and HFCS) or high-fat diet (FD and HFCS/FD) for 14 weeks as described in the 'Experimental methods' section. Values are means + SD (n 10).

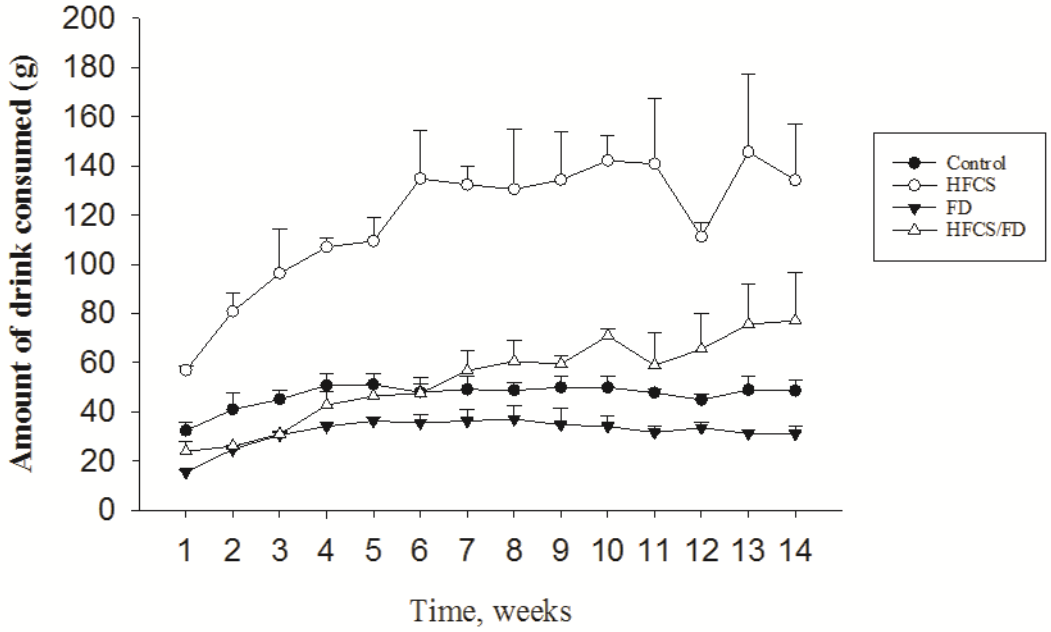


Figure 3.3: Average consumed fluid volume per rat from each group. Groups were on either: Normal drinking water (Control and FD groups) or HFCS (HFCS and HFCS/FD groups) for 14 weeks as described in the 'Experimental methods' section. Values are means + SD (n 10).

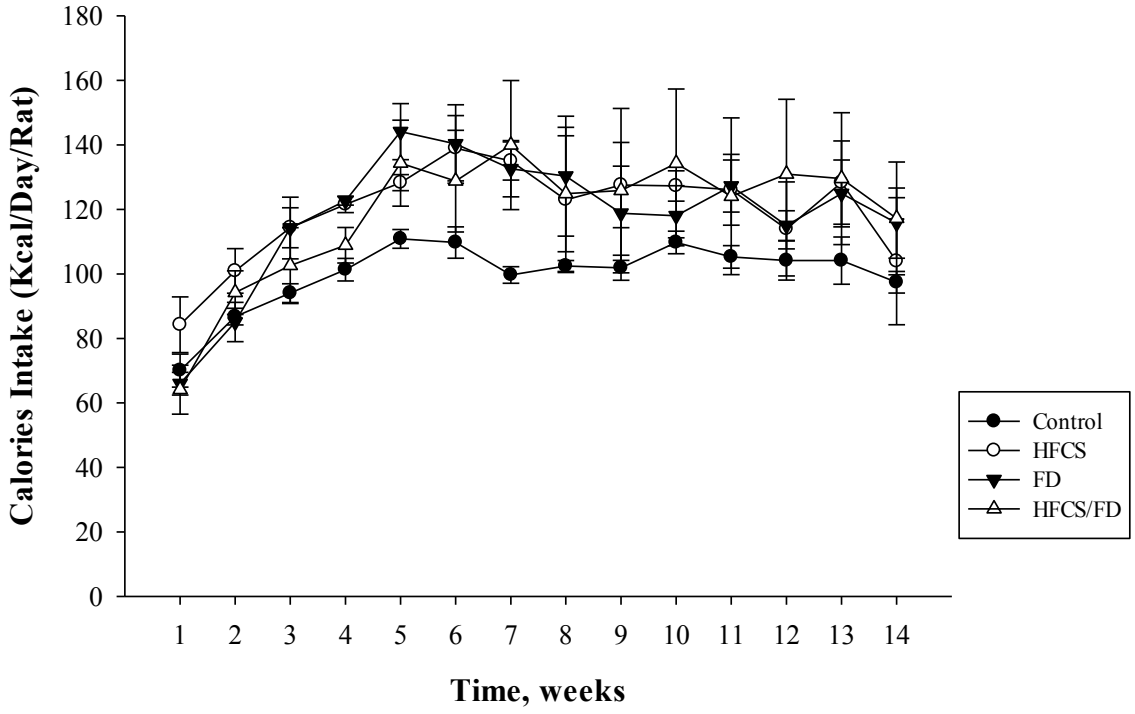


Figure 3.4: Daily caloric intake (Kcal/day/rat) of all groups. Rats were fed either: (Control) standard diet + normal drinking water, (HFCS) standard diet + HFCS, (FD) high-fat diet + normal drinking water or (HFCS/FD) high-fat diet + HFCS for 14 weeks as described in the 'Experimental methods' section. Values are means ± SD (n = 10).

3.2. Plasma Biochemical results

3.2.1. Changes in Plasma Levels of Cholesterol, HDL and Triglyceride

To investigate whether high caloric diet was associated with changes in lipid and lipoprotein concentrations, the plasma samples from fasted rats were assayed for cholesterol, HDL and triglyceride. Both FD and HFCS/FD groups displayed significant increases in cholesterol plasma levels compared to the control group. The HFCS group showed a trend toward increase in plasma cholesterol levels (Fig. 3.5.A). All three groups on high caloric diet showed numerical increases in the mean plasma levels of triglyceride compared to the control group, but this was significantly higher only for the HFCS and HFCS/FD groups (Fig. 3.5.B). HDL was significantly higher only in the FD groups (Fig. 3.5.C).

3.2.2. Changes in Plasma Levels of Glucose and Insulin

To investigate whether the rats on high caloric diets developed hyperglycemia and/or hyperinsulinemia, the fasted plasma levels of glucose and insulin were measured. Blood glucose levels were significantly higher only in the HFCS/FD group (Fig. 3.6.A). Unlike glucose, for each of the high caloric diet groups there was a significant increase (approximately 3-fold) in plasma insulin levels compared to control rats (Fig. 3.6.B).

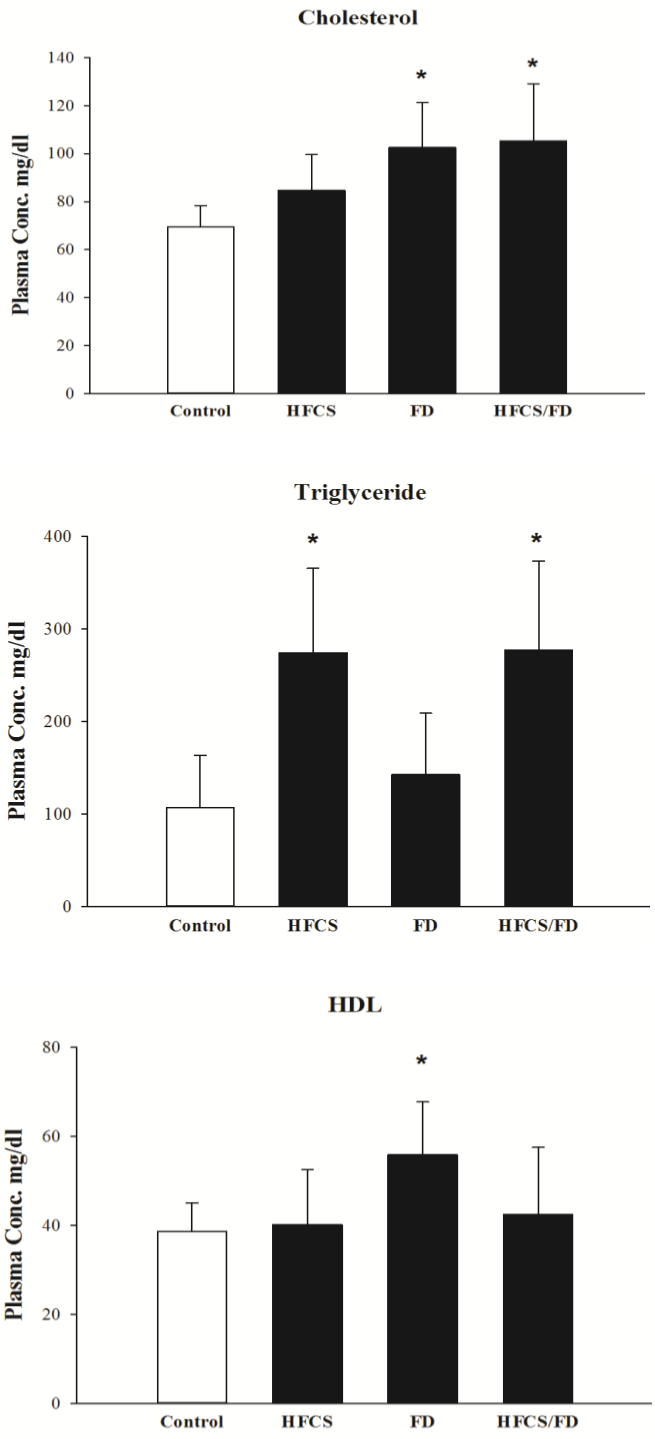


Figure 3.5: Cholesterol (Top panel), **Triglyceride** (middle panel), **HDL** (lower panel) plasma concentrations (mean + SD) 14 weeks after start of experiment. Data were compiled from 40 rats (10/group). Comparisons were made between the control and other groups on high caloric diet. * denotes statistically significant using one-way ANOVA followed by post-hoc Tukey test, $P < 0.05$.

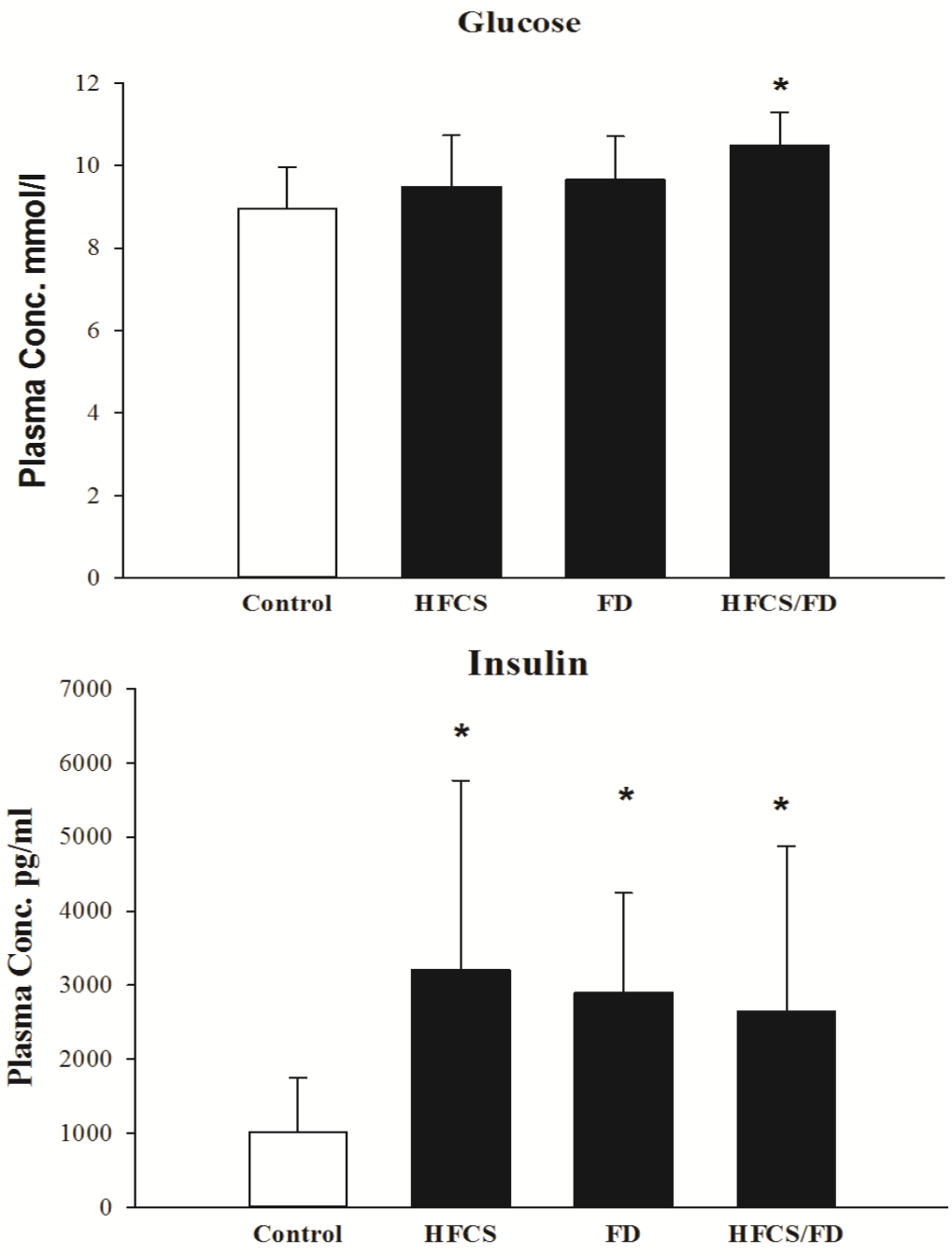


Figure 3.6: Glucose (Top panel) and **Insulin** (Lower panel) plasma concentrations (mean + SD) 14 weeks after start of experiment. Data were compiled from 40 rats (10/group). Comparisons were made between the control and other groups on high caloric diet. * Denotes statistically significant using one-way ANOVA followed by post-hoc Tukey test, $P < 0.05$.

3.2.3. Changes in Leptin and other plasma mediators

To investigate whether increased adiposity can change the plasma levels of leptin and other proteins involved in energy homeostasis, blood pressure, immunity and inflammations, therefore, concentrations of leptin, adiponectin, amylin (active), GIP, GLP-1 (active), ghrelin, PYY, and TNF-α were measured in fasted plasma of 40 rats.

Plasma leptin levels were significantly higher in all high caloric diet-fed rats. Compared to the control rats, there were 2.7-, 4.2- and 3.4-fold increases in leptin concentrations in HFCS, FD and HFCS/FD respectively (Fig.3.7). GIP was significantly higher only in FD group. No significant differences were found between the control group and the other groups in the plasma levels of GLP, adiponectin, amylin (active), ghrelin, PYY, and TNFα. (Tab. 3.1)

3.2.4. Changes in plasma levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST)

To investigate whether liver damage can be developed by feeding rats high caloric diet for 14 weeks, the ALT and AST plasma levels analysis were performed. There were non-significant changes in plasma levels of ALT in rats of FD and HFCS/FD groups. In contrast, ALT was significantly lower in HFCS rats (Fig. 3.8.A). In contrast, no significant changes were found in AST levels between control and other groups (Fig. 3.8.B).

Table 3.1: Concentrations of some plasma parameters in rats of different groups 14 weeks after start of experiment. Data were compiled from 40 rats (10/group). Comparisons were made between the control and other groups on high caloric diet. All the measured parameters in this table showed non-significant changes compared to control except for GIP plasma level in FD group.

PLASMA PARAMETER	CONTROL	HFCS	FD	HFCS/FD
GIP, pg/mL Geometric mean (cv%)	101 (47.9)	83.4 (76.9)	241.72 (81.36)*	156.67 (57.72)
Amylin, pg/mL Geometric mean (cv%)	71.69 (8.38)	74.31 (8.99)	78.74 (4.71)	75.74 (12.92)
GLP1, pg/mL Geometric mean (cv%)	61.29 (171.91)	56.81 (47.97)	53.14 (126.1)	82.13 (75.12)
Ghrelin, pg/mL Geometric mean (cv%)	22.37 (62.93)	20.09 (54.33)	21.61 (66.89)	19.05 (89.51)
TNF, pg/mL Geometric mean (cv%)	105.63 (7.34)	105.71 (12.69)	95.57 (13.26	101.61 (19.21)
PYY, pg/mL Geometric mean (cv%)	67.62 (31.08)	60 (5.79)	62.33 (13.53)	65.63 (10.92)
Adiponectin, µg/mL Geometric mean (cv%)	13.3 (23)	16.4 (39)	11.4 (38)	12.5 (40)

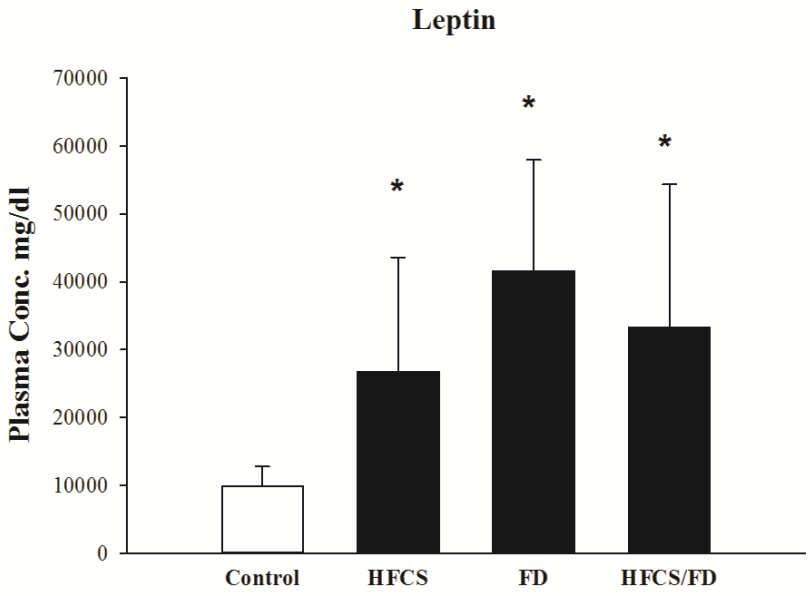


Figure 3.7: Leptin plasma concentrations (mean + SD) 14 weeks after start of experiment. Data were compiled from 40 rats (10/group). Comparisons were made between the control and other groups on high caloric diet. * Statistically significant using one-way ANOVA followed by post-hoc Tukey test, $P < 0.05$.

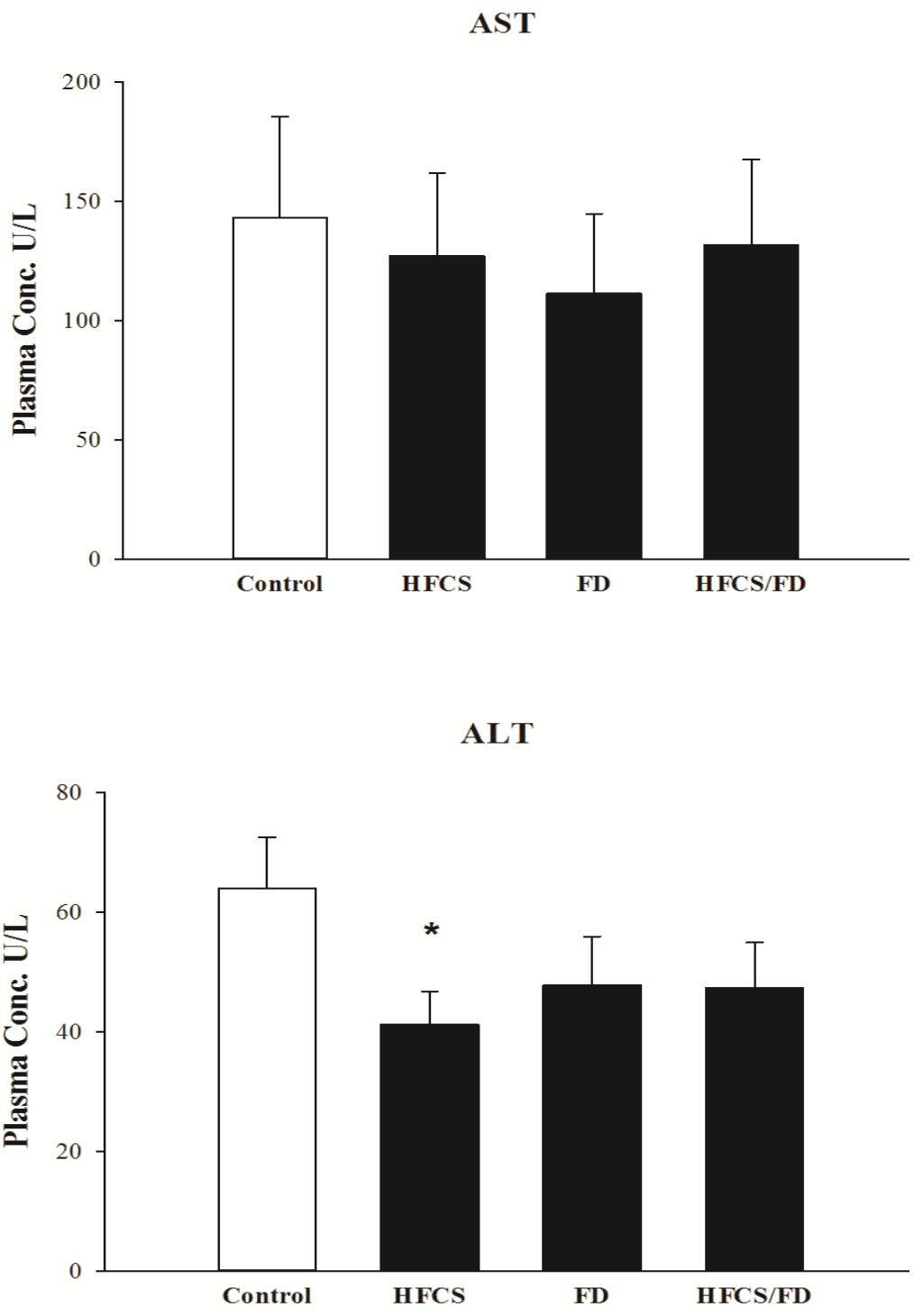


Figure 3.8: Liver function enzymes: **AST** (Top panel) and **ALT** (Lower panel) plasma concentrations (mean + SD) 14 weeks after start of experiment. Data were compiled from 40 rats (10/group). Comparisons were made between the control and other groups on high caloric diet. * Denotes statistically significant using one-way ANOVA followed by post-hoc Tukey test, $P < 0.05$.

3.3. Real Time PCR and Western Blotting Results

3.3.1. Liver tissues

3.3.1.1. CYPs and UGTs expression

3.3.1.1.1. Real time PCR

To examine the effect of high fat diet and/or HFCS on CYP450s expression, total mRNA was extracted from the rat livers of control and high caloric diet groups. Thereafter, the expressions of different CYP450 genes were measured.

It was found that high fat diet and HFCS caused significant decreases in *Cyp3a2* gene expression by 3-5 fold in the liver tissues of all high caloric diet groups compare to control (Fig 3.9.A). All high caloric diet groups showed non-significant changes in gene expression of *Cyp3a1* (Fig 3.10.A) and *Cyp1a1* (Fig 3.12.A). In case of *Cyp2c11*, there were trend toward decrease in all high caloric diet groups compared to control group (Fig 3.11.A). In terms of *Udpgt2b1*, there were significant decreases in all high caloric diet groups compare to control. In contrast, *Udpgt1a1* showed trend toward decrease in high caloric diet groups but did not reach significant (Fig 3.13).

3.3.1.1.2. Western Blot Results

To investigate whether the changes in P450 due to high fat diet and/or HFCS, the microsomal proteins were prepared from rats' liver of control and other groups. Thereafter, CYP3A1, CYP3A2, CYP2C11 and CYP1A1 protein levels were determined using western blot analysis. Compared to the control group, there were significant decreases in protein levels of CYP3A2 (Fig 3.9.B), CYP3A1 (3.10.B) and CYP2C11 (Fig

3.11.B) in both FD and HFCS/FD groups. HFCS group showed trend toward decrease in these proteins but was not significant. CYP1A1 demonstrated non-significant changes between groups (Fig 3.12.B).

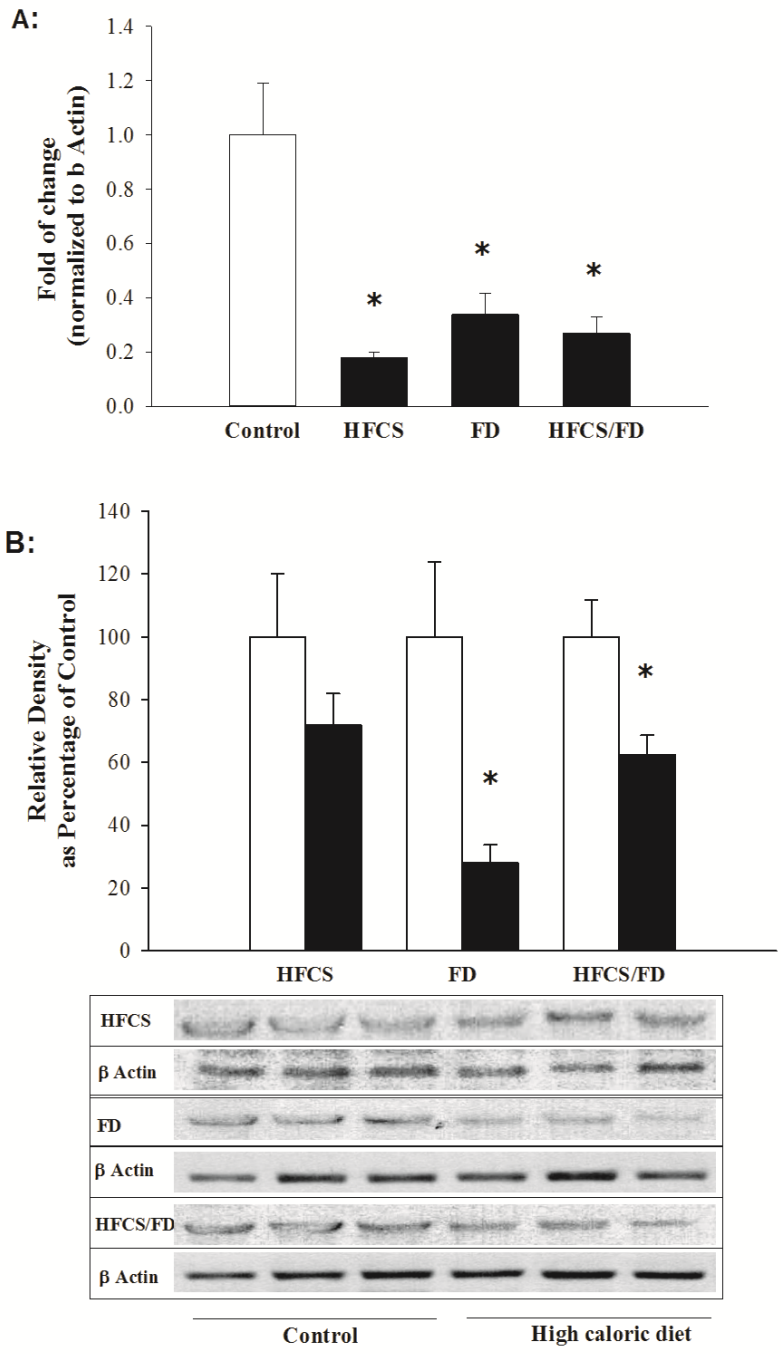


Figure 3.9: Effect of high caloric diet on metabolizing enzyme CYP3A2 mRNA/ protein expression in rat liver tissue. (A) *Cyp3a2* mRNA levels were quantified using RT-PCR and normalized to β actin. Duplicate reactions were performed for each experiment. (B) CYP3A2 protein was detected using the enhanced chemiluminescence method. The intensity of bands was normalized to β actin signals. One of three representative experiments is shown. Data are presented in fold of control (mean + S.E.M., n = 4-8). * P <0.05 compared to control using unpaired Student t-test.

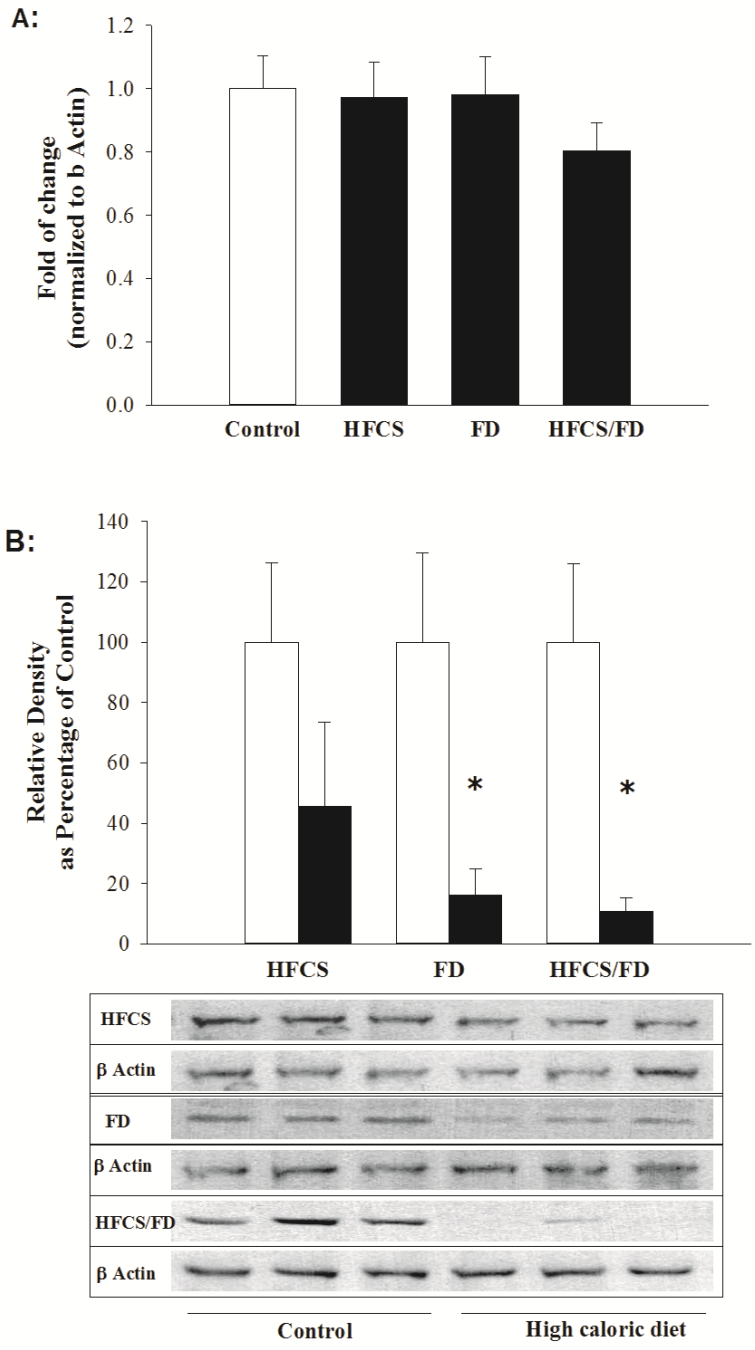


Figure 3.10: Effect of high caloric diet on metabolizing enzyme CYP3A1 mRNA/ protein expression in rat liver tissue. (A) *Cyp3a1* mRNA levels were quantified using RT-PCR and normalized to β actin. Duplicate reactions were performed for each experiment. **(B)** CYP3A1 protein was detected using the enhanced chemiluminescence method. The intensity of bands was normalized to β actin signals. One of three representative experiments is shown. Data are presented in fold of control (mean + S.E.M., n = 4-8). * P <0.05 compared to control using unpaired Student t-test.

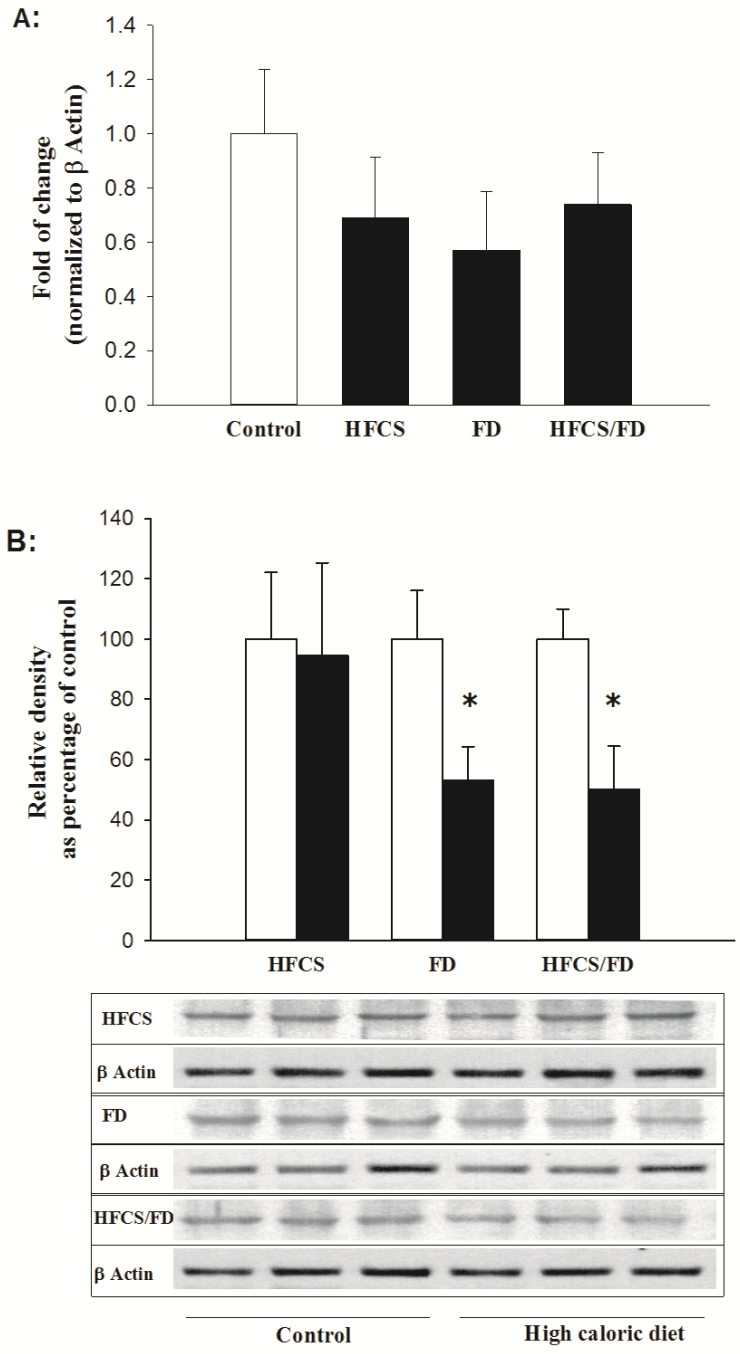


Figure 3.11: Effect of high caloric diet on metabolizing enzyme CYP2C11 mRNA/ protein expression in rat liver tissue. (A) *Cyp2c11* mRNA levels were quantified using RT-PCR and normalized to β actin. Duplicate reactions were performed for each experiment. (B) CYP2C11 protein was detected using the enhanced chemiluminescence method. The intensity of bands was normalized to β actin signals. One of three representative experiments is shown. Data are presented in fold of control (mean + S.E.M., n = 4-8). * P <0.05 compared to control using unpaired Student t-test.

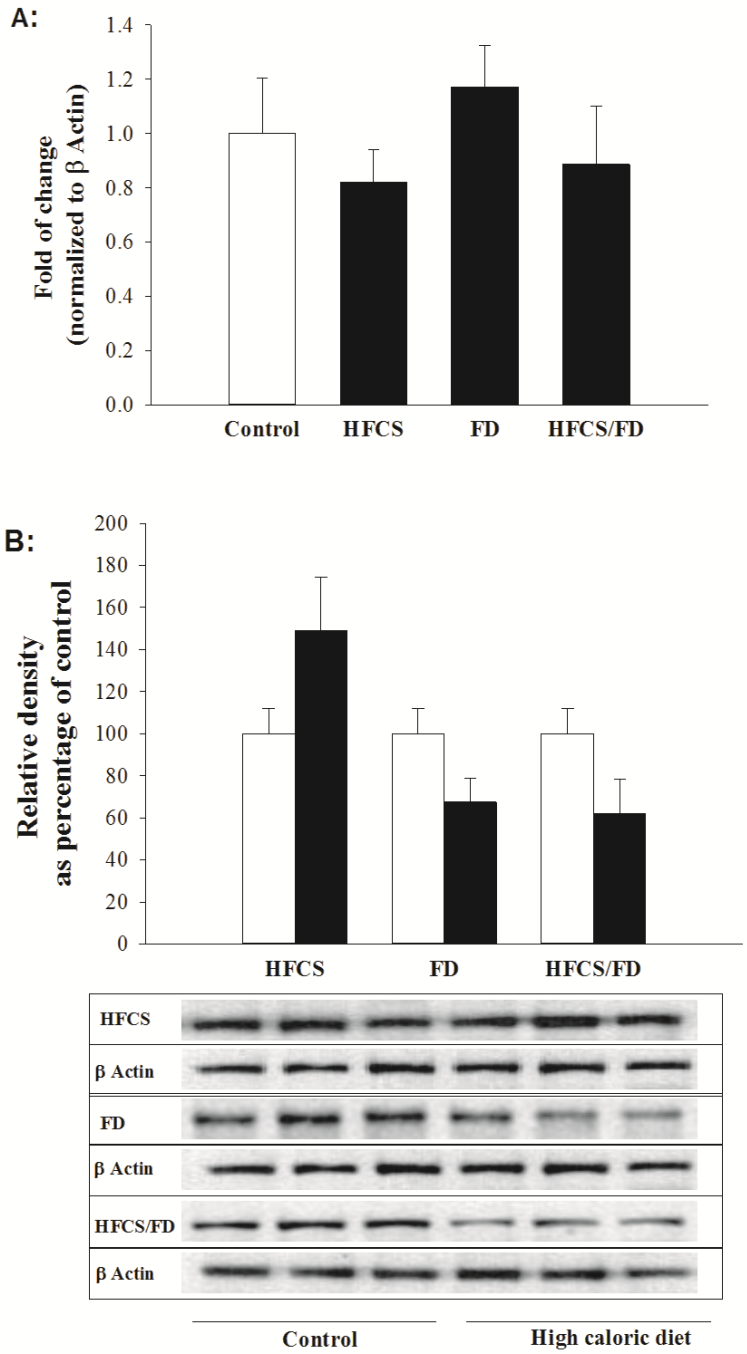


Figure 3.12: Effect of high caloric diet on metabolizing enzyme CYP1A1 mRNA/ protein expression in rat liver tissue. (A) *Cyp1a1* mRNA levels were quantified using RT-PCR and normalized to β actin. Duplicate reactions were performed for each experiment. (B) CYP1A1 protein was detected using the enhanced chemiluminescence method. The intensity of bands was normalized to β actin signals. One of three representative experiments is shown. Data are presented in fold of control (mean + S.E.M., n = 4-8). Compared to control no significant differences were found (unpaired Student t-test).

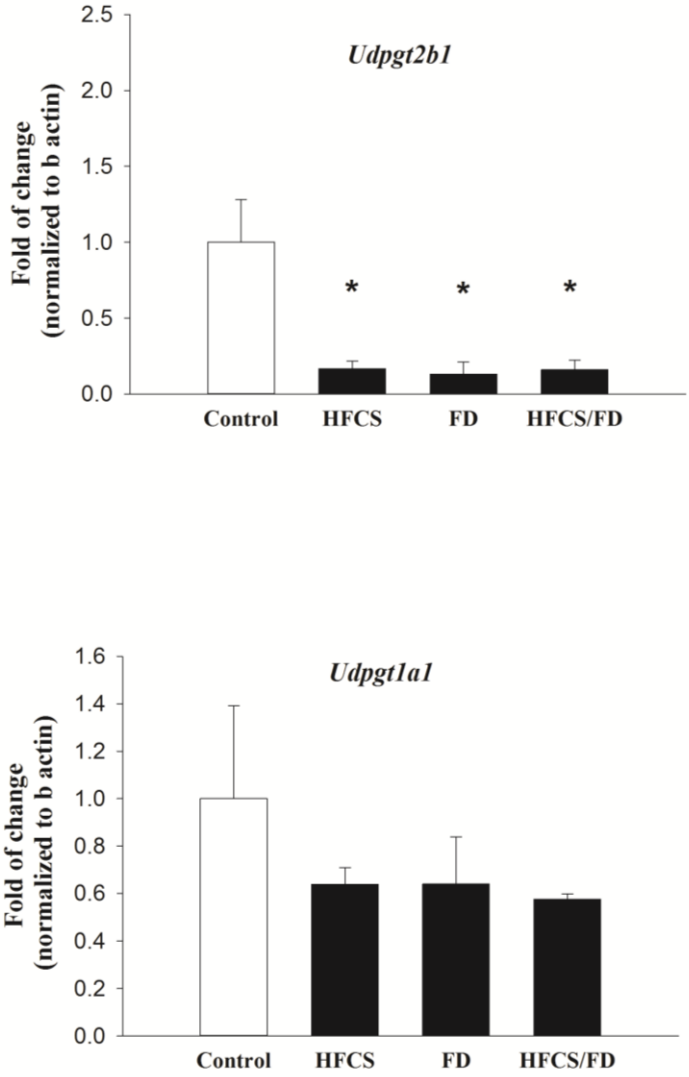


Figure 3.13: Effect of high caloric diet on metabolizing enzyme mRNA of Udpgt2b1 and 1a1 in rat liver tissue. The mRNA levels for Udpgt2b1 (Top panel) and Udpgt1a1 (Lower panel) were quantified using RT-PCR and normalized to β actin. Duplicate reactions were performed for each experiment. Data are presented in fold of control (mean + S.E.M., n = 4-8).

3.3.1.2 Transporters protein expression

3.3.1.2.1 Real time PCR

To study the effect of high caloric diet on efflux transporters (MATE1 and MDR1) and influx transporter (OCT1) expressions, their gene expressions in liver tissues were measured. All high caloric diet groups displayed non-significant changes in gene expression levels of *Mate1* (Fig 3.14.A), *Mdr1* (Fig 3.15.A) and *Oct1* (3.16.A) compared to that of control group.

3.3.1.2.2 Western Blot Results

Liver protein levels of MATE1, MDR1 and OCT1 were measured for all groups. The expression of MATE1 displayed significant inhibition only in FD and HFCS/FD groups compare to control (Fig 3.14.B). MDR1 displayed general non-significant changes in its expression in all high caloric diet groups (Fig 3.15.B). HFCS/FD group showed a significant inhibition in the expression of OCT1 transporter, whereas the expressions in other groups did not show any significant differences compared to control group (Fig 3.16.B).

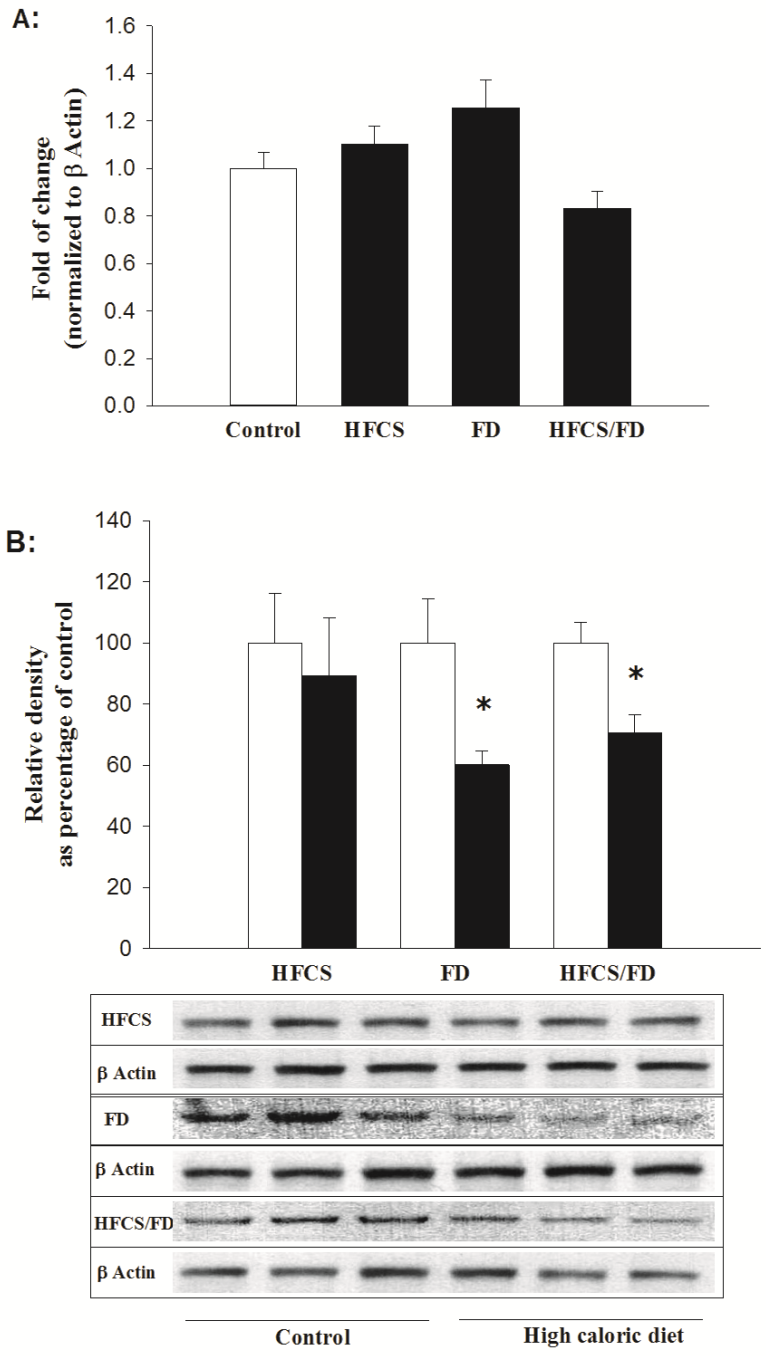


Figure 3.14: Effect of high caloric diet on MATE1 mRNA/ protein expression in rat liver tissue. (A) *Mate1* mRNA levels were quantified using RT-PCR and normalized to βactin. Duplicate reactions were performed for each experiment. (B) MATE1 protein was detected using the enhanced chemiluminescence method. The intensity of bands was normalized to β actin signals. One of three representative experiments is shown. Data are presented in fold of control (mean + S.E.M., n = 4-8). * P <0.05 compared to control using unpaired Student t-test.

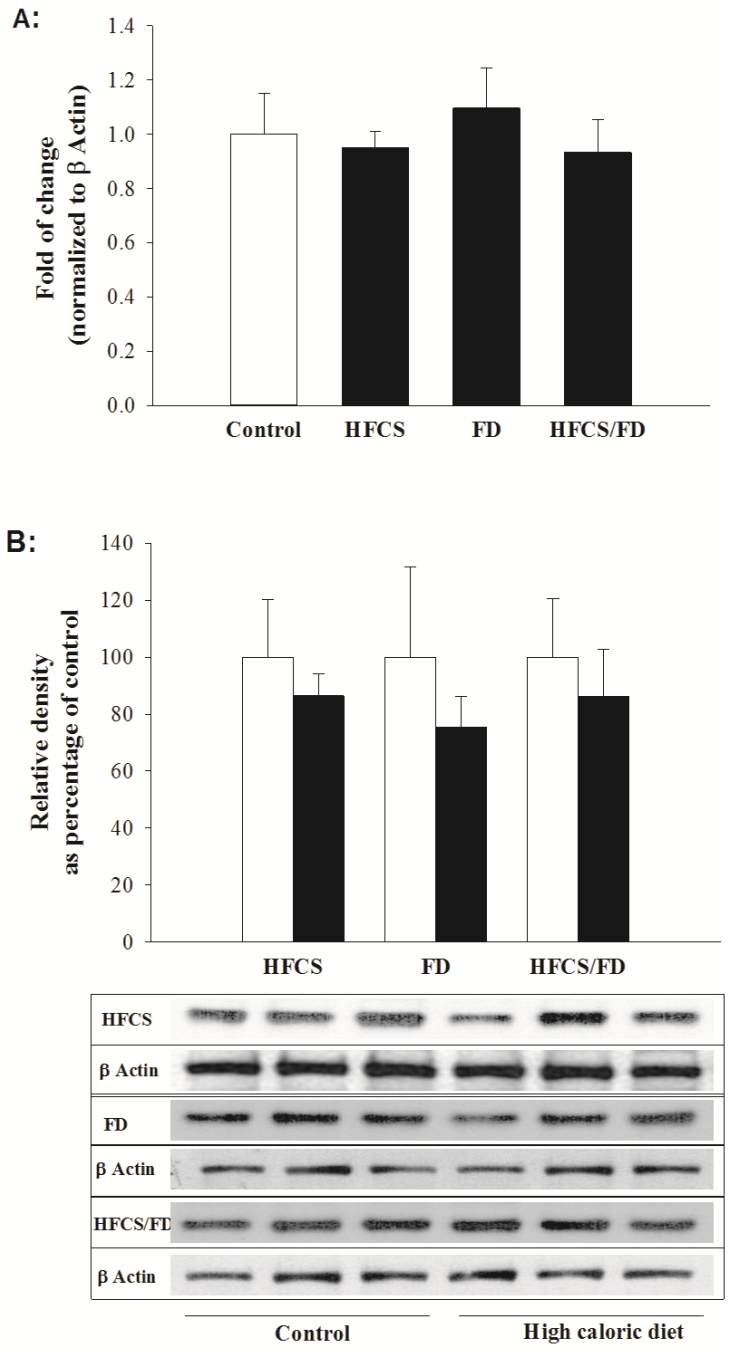


Figure 3.15: Effect of high caloric diet on MDR1 mRNA/ protein expression in rat liver tissue. (A) *Mdr1* mRNA levels were quantified using RT-PCR and normalized to βactin. Duplicate reactions were performed for each experiment. (B) MDR1 protein was detected using the enhanced chemiluminescence method. The intensity of bands was normalized to β actin signals. One of three representative experiments is shown. Data are presented in fold of control (mean + S.E.M., n = 4-8). Compared to control no significant differences were found (unpaired Student t-test).

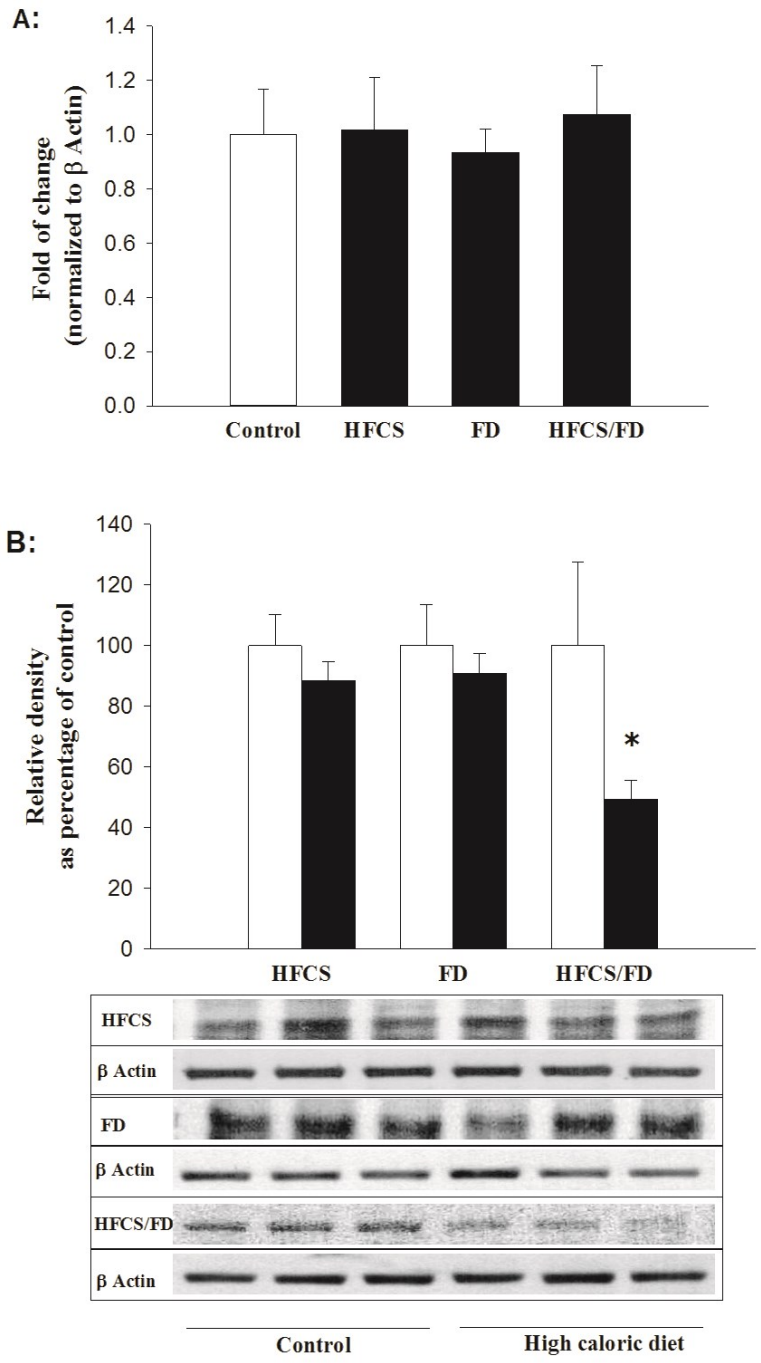


Figure 3.16: Effect of high caloric diet on OCT1 mRNA/ protein expression in rat liver tissue. (A) *Oct1* mRNA levels were quantified using RT-PCR and normalized to βactin. Duplicate reactions were performed for each experiment. (B) OCT1 protein was detected using the enhanced chemiluminescence method. The intensity of bands was normalized to β actin signals. One of three representative experiments is shown. Data are presented in fold of control (mean + S.E.M., n = 4-8). * P <0.05 compared to control using unpaired Student t-test.

3.3.2 Kidney tissues

3.3.2.1 Real time PCR

To determine the role of high-caloric diet in the regulation of genes associated with drug transportation in kidneys, total RNA was extracted from the rat kidneys of all groups. Thereafter, the genes expression of *Oct1, Oct2, Mate1*, and *Mdr1* were measured.

Our finding demonstrated non-significant changes in gene expression levels of *Oct1* (Fig 3.17.A), *Oct2* (3.18.A), *Mate1* (Fig 3.19.A) and *Mdr1* (Fig 3.20.A) compared to that of control group.

3.3.2.2 Western Blot Results

In kidney of all groups the OCT1, OCT2, MATE1 and MDR1 protein levels were determined. Only HFCS/FD group showed significant inhibition in OCT1 protein expression compared to control group (Fig 3.17.B). In case of OCT2, all groups on high caloric diet displayed inhibition in OCT2 protein expression but were significant in case of FD and HFCS/FD groups only (Fig 3.18.B). Compare to control group, all high caloric diet groups showed non-significant changes in protein expressions of MATE1 (Fig 3.19.B) and MDR1 (Fig 3.20.B).

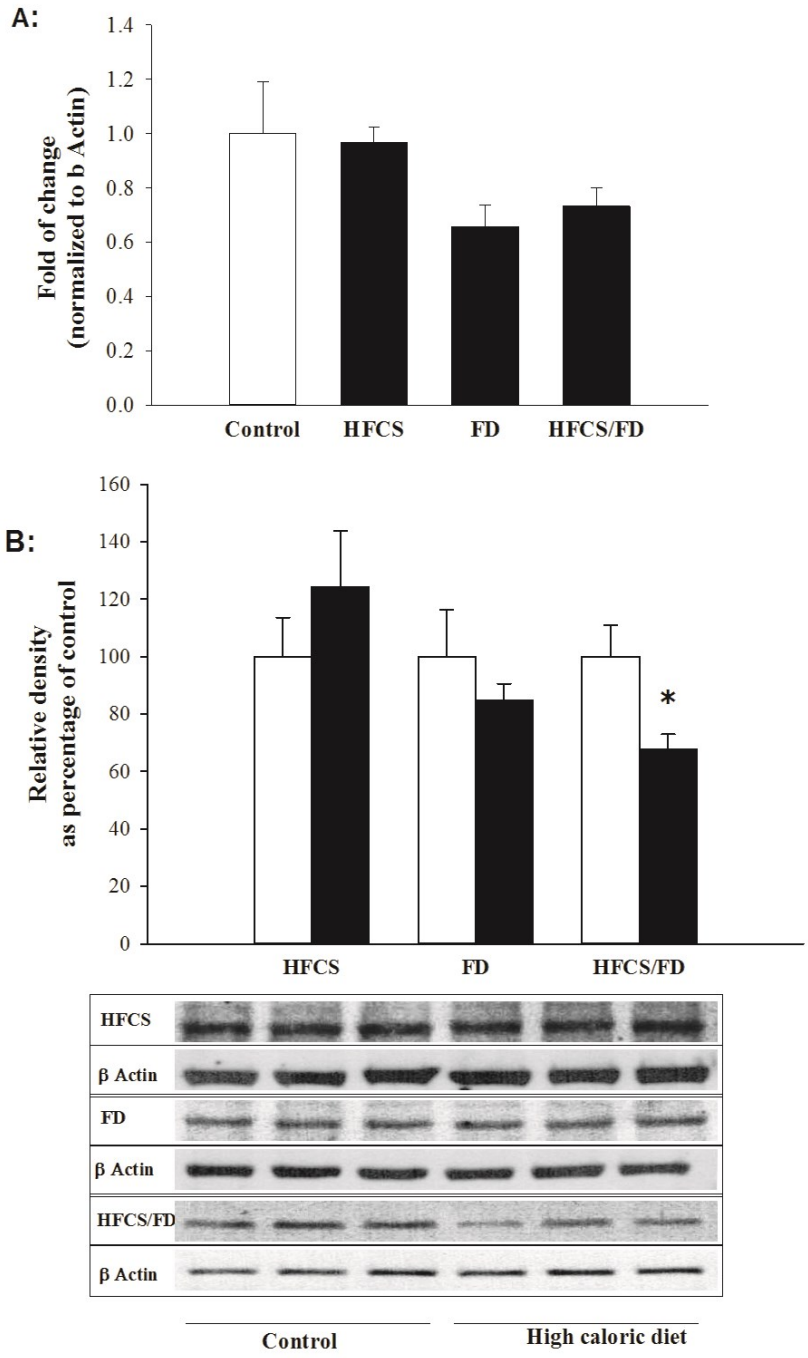


Figure 3.17: Effect of high caloric diet on OCT1 mRNA/ protein expression in rat kidney tissue. (A) *Oct1* mRNA levels were quantified using RT-PCR and normalized to βactin. Duplicate reactions were performed for each experiment. (B) OCT1 protein was detected using the enhanced chemiluminescence method. The intensity of bands was normalized to β actin signals. One of three representative experiments is shown. Data are presented in fold of control (mean + S.E.M., n = 4-8). * P <0.05 compared to control using unpaired Student t-test.

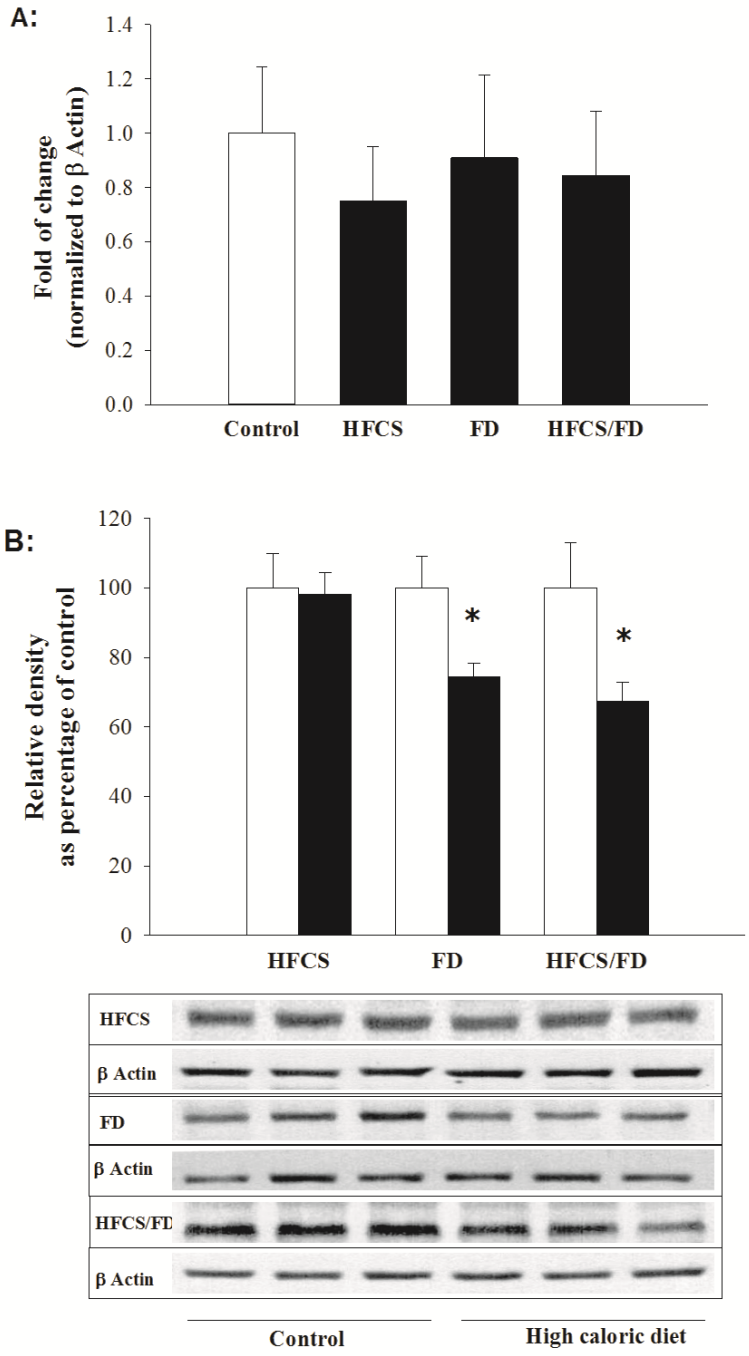


Figure 3.18: Effect of high caloric diet on OCT2 mRNA/ protein expression in rat kidney tissue. (A) *Oct2* mRNA levels were quantified using RT-PCR and normalized to βactin. Duplicate reactions were performed for each experiment. (B) OCT2 protein was detected using the enhanced chemiluminescence method. The intensity of bands was normalized to β actin signals. One of three representative experiments is shown. Data are presented in fold of control (mean + S.E.M., n = 4-8). * P <0.05 compared to control using unpaired Student t-test.

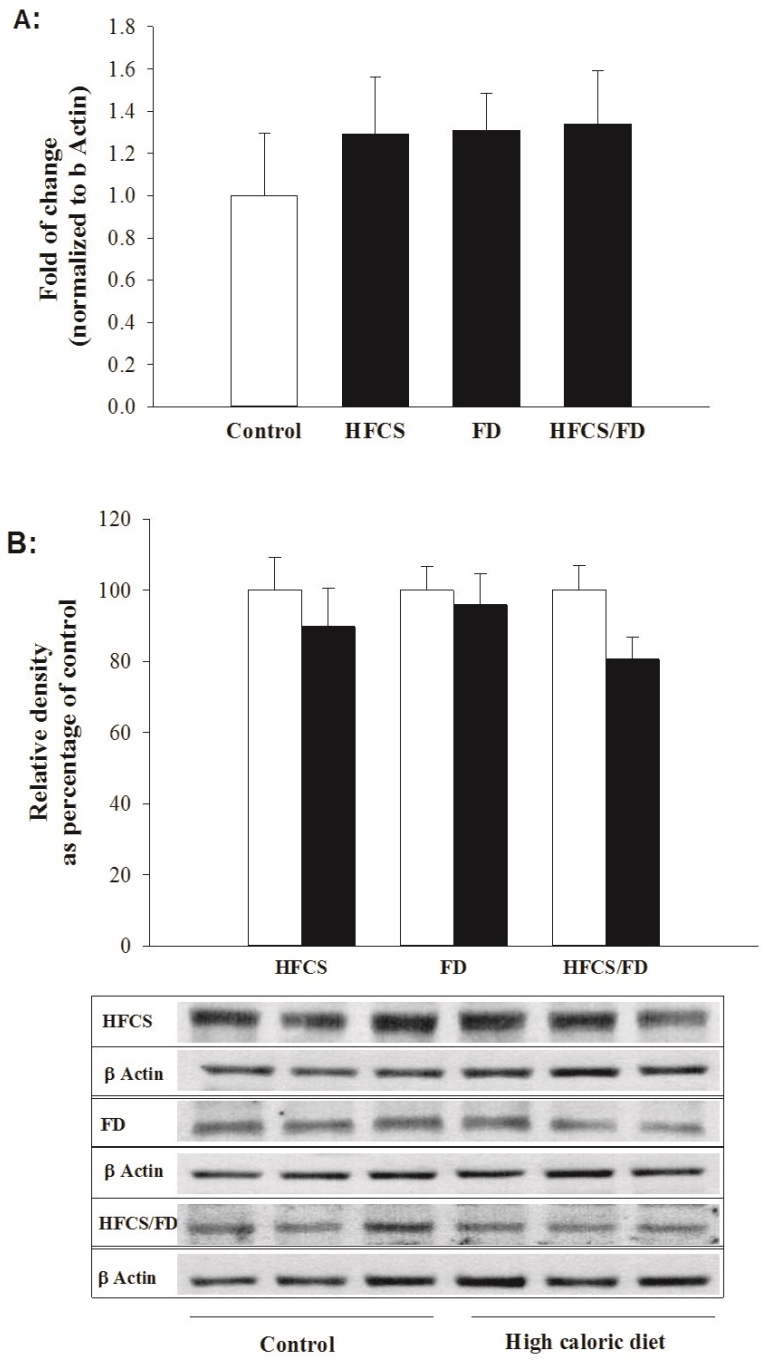


Figure 3.19: Effect of high caloric diet on MATE1 mRNA/ protein expression in rat kidney tissue. (A) *Mate1* mRNA levels were quantified using RT-PCR and normalized to βactin. Duplicate reactions were performed for each experiment. (B) MATE1 protein was detected using the enhanced chemiluminescence method. The intensity of bands was normalized to β actin signals. One of three representative experiments is shown. Data are presented in fold of control (mean + S.E.M., n = 4-8). Compared to control no significant differences were found (unpaired Student t-test).

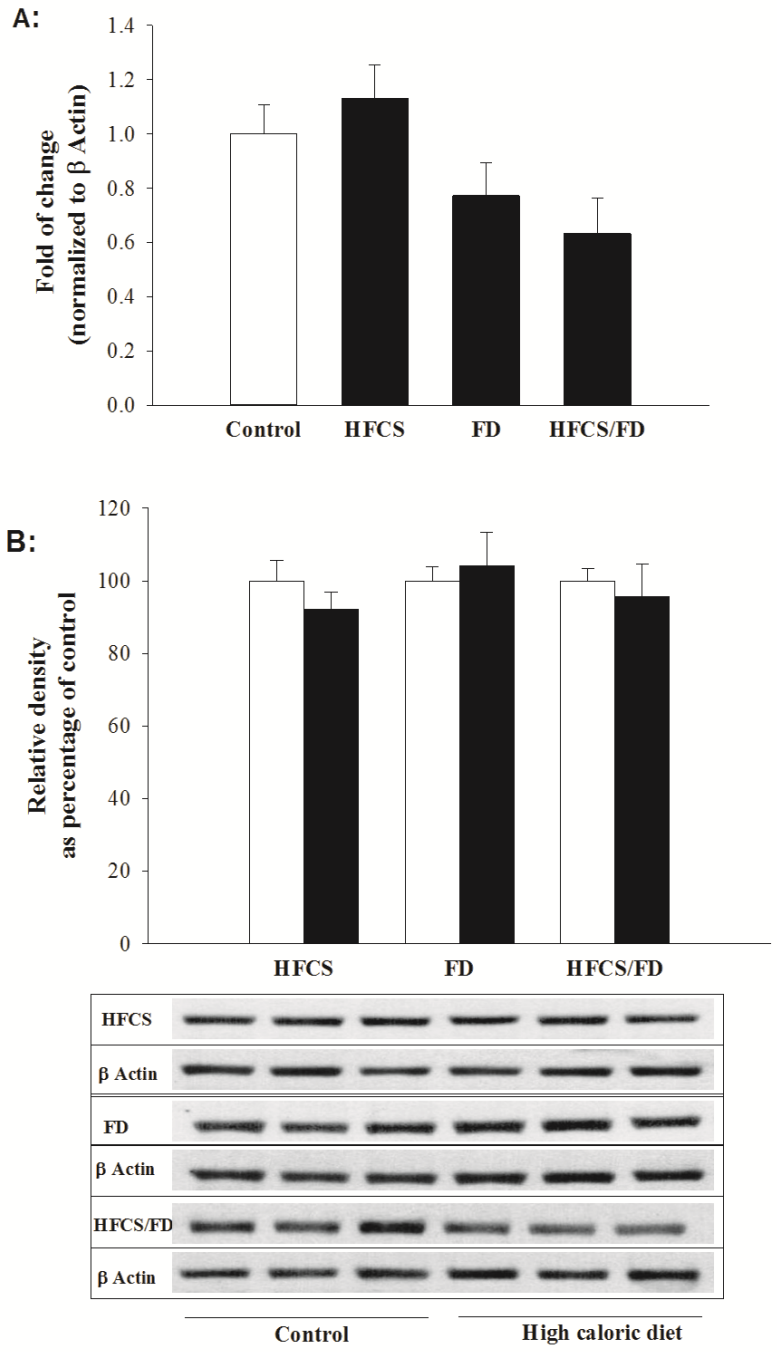


Figure 3.20: Effect of high caloric diet on MDR1 mRNA/ protein expression in rat kidney tissue. (A) *Mdr1* mRNA levels were quantified using RT-PCR and normalized to β actin. Duplicate reactions were performed for each experiment. (B) MDR1 protein was detected using the enhanced chemiluminescence method. The intensity of bands was normalized to β actin signals. One of three representative experiments is shown. Data are presented in fold of control (mean + S.E.M., n = 4-8). Compared to control no significant differences were found (unpaired Student t-test).

3.4 Biodistribution Study Results

3.4.1 Biodistribution of AM after single oral dose of AM 25 mg/Kg

3.4.1.1 AM Plasma Concentration

Compared to the control group, the plasma concentrations of HFCS/FD were higher at all measured time points but this elevation was only significant at 3 h post dose. Higher Cmax of AM was found in the HFCS/FD group compare to control (1419 vs. 972 ng/mL) but did not reach significance. In both groups, AM reached Cmax after 1 hour (first time point of AM concentration vs. time curve) of AM single oral dose. The plasma concentrations vs. time curve showed a declined in AM levels over the first 9 hours, after which the postdistributive phase appeared to be reached (Fig 3.21).

3.4.1.2 AM Tissue Distribution

In the tissues analyzed for drug content, AM was detected in all specimens at quantifiable concentrations. In those specimens in which AM was detected, the order in mean AUC from highest to lowest in the both groups were liver > lung > heart > plasma. (Tab 3.2, Fig 3.22). For liver tissue, the mean AUC_{0-24} was 1.52-fold higher in the HFCS/FD compared to control rats. However, AUC_{0-24} in both lung and heart tissues were almost the same in both groups.

Although the mean value is reported, statistical testing could not be performed on the differences in Kp based on AUC data because of the study design (1 timed sample per animal). During the postdistributive phase of the concentration vs. time curves, which appeared from 9 h onward for AM, the mean postdistributive Kp (ie, 9-24 hours

postdose) for each tissue sample was compared. A higher Kp was noted for control than for HFCS/FD animals in both lung and heart tissues but the difference was not significant. However, in the liver Kp was significantly higher in HFCS/FD compared to control rats (Fig 3.23).

Table 3.2: Area under the plasma and tissue concentrations versus time curve (±SD) for AM (ng×hr/mL or g) after oral administration of AM 25 mg/kg to rats (n=16/group). * Denotes significant difference between two groups (using Bailer's method).

Specimen	Group	
	Control	**HFCS/FD**
Plasma	8260 ± 598	12471 ± 1397 *
Liver	350520 ± 36362	532182 ± 76448
Heart	142818 ± 13724	133118 ± 8556
Lung	265553 ± 15717	252576 ± 26954

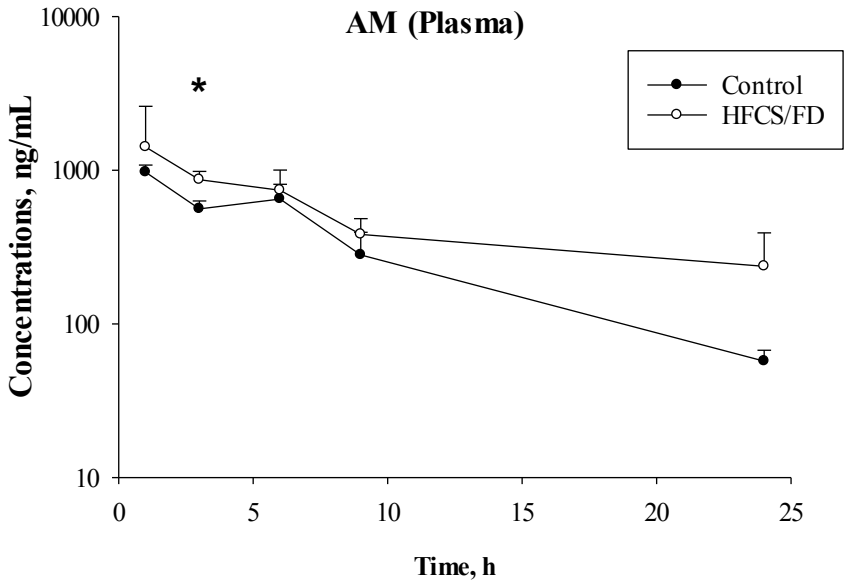
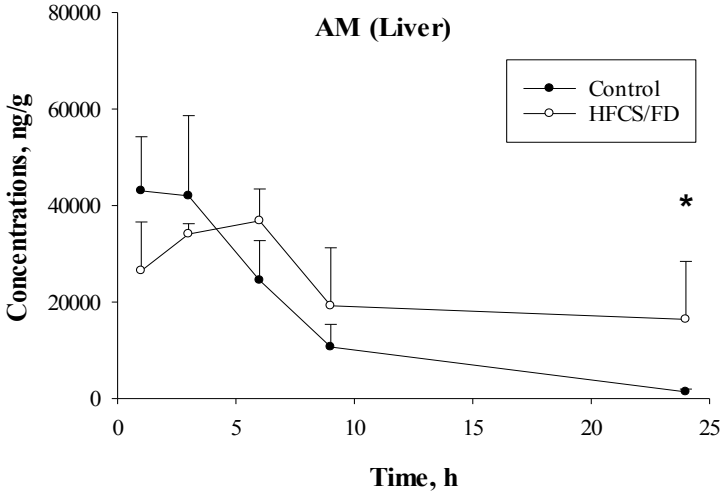
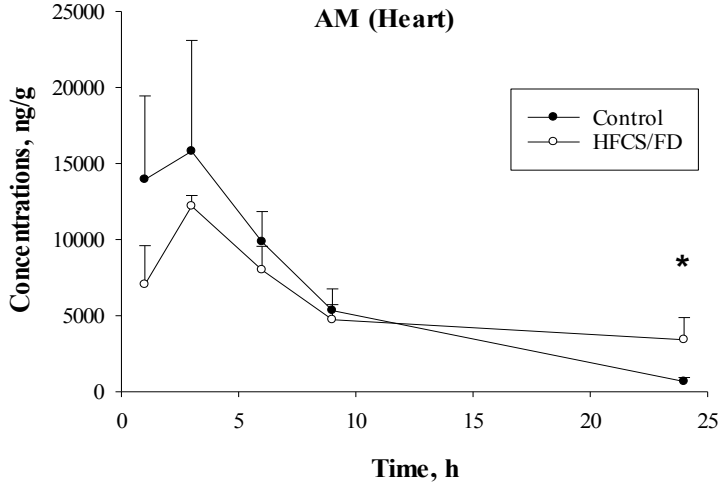


Figure 3.21: Concentration vs. time profiles of AM in the plasma following oral administration of 25mg/kg AM. All data are presented as mean + SD (n =16/group). * Denotes a significant difference in AM concentrations at that time point between control and HFCS/FD groups ($p<0.05$, unpaired t-test).





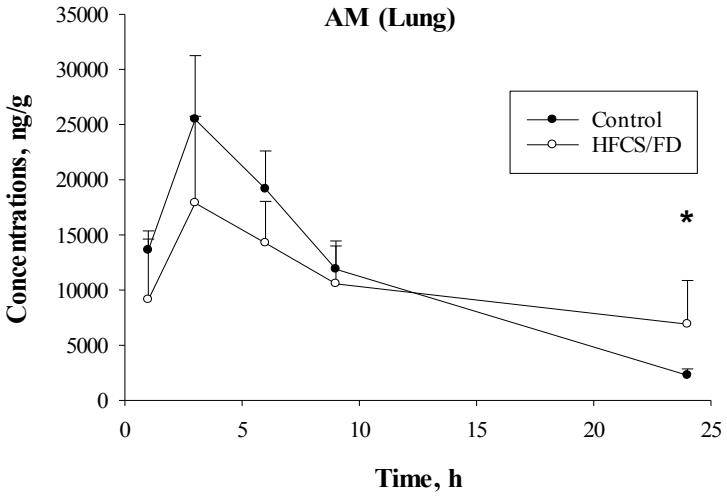


Figure 3.22: Concentration vs. time profiles of AM in Liver (Top panel), Heart (Middle panel) and Lung (Lower panel) following oral administration of AM 25mg/kg. All data are presented as mean + SD (n =16/group). * Denotes a significant difference in AM concentrations at that time point between control and HFCS/FD groups ($p<0.05$, unpaired t-test).

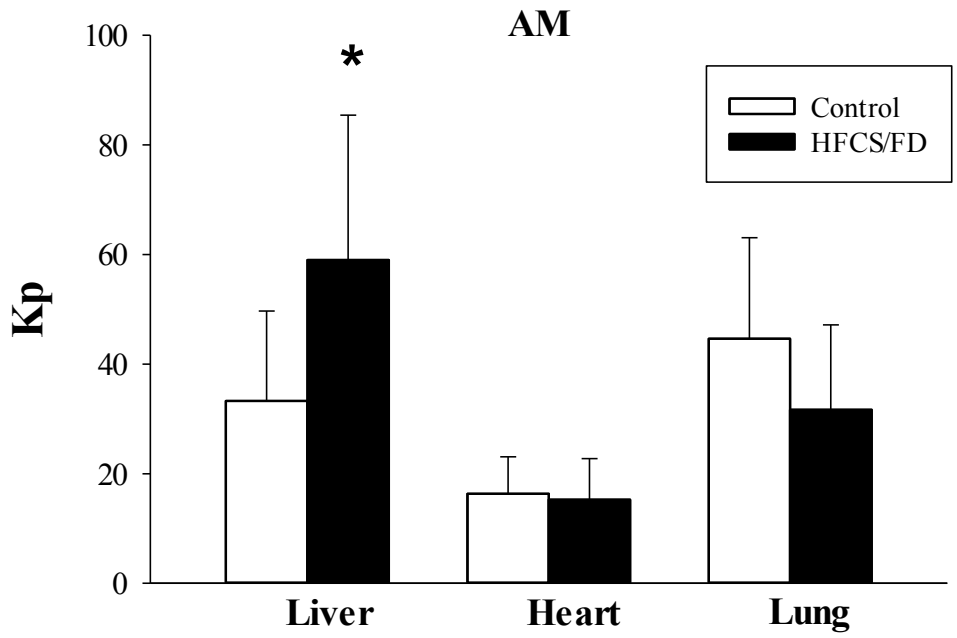


Figure 3.23: Kp vs. time profiles for AM after oral administration of AM 25mg/kg. The data presents Liver, Heart and Lung to plasma ratios for AM. All data presented as mean + SD (n = 16/group). * Denotes significant difference in Kp of AM between control and HFCS/FD groups ($p<0.05$, unpaired t-test).

3.4.2 Biodistribution of DEA after single oral dose of AM 25mg/Kg

3.4.2.1 DEA Plasma Concentration

Plasma concentrations of DEA were at its maximum values after 6 h of the AM dose. DEA plasma concentrations of HFCS/FD were lower than that of control group at 1, 3, 6, 9 hours post dose but these decreases were not significant. After 24 hours post dose the plasma concentration was non-significantly higher in HFCS/FD compared to that of control (Tab 3.3, Fig 3.24). The plasma concentrations vs. time curve for both groups showed a gradual increases in DEA concentrations to reach its maximum at 6 hours post dose. The Cmax of DEA was found to be decreased in the HFCS/FD group (139 ng/ml) compare to control (197 ng/ml), but this decrease was not significant. After that, DEA concentrations start to decrease up to 9 hours after dose, after which the postdistributive phase was reached (Fig 3.24).

3.4.2.2 DEA Tissue Distribution

DEA was detected in all specimens at quantifiable concentrations. In those specimens in which DEA was detected, the order in mean AUC from highest to lowest in control group were lung > liver > heart > plasma. However, in the HFCS/FD group AUC were ranked in order of liver > lung > heart > plasma (Tab 3.3). The mean AUC_{0-24} of control group was higher than that of HFCS/FD group in both lung and heart tissues by 1.27- and 1.18-fold respectively. In contrast, AUC_{0-24} was 1.28-fold higher in liver tissue of the HFCS/FD rats compared to control rats (Fig 3.25). The postdistributive phase of the concentration vs. time curves appeared from 9 h onward for DEA (same for AM). The mean postdistributive Kp 9-24 hours post-dose for each tissue sample was compared (Fig

3.26), a higher Kp was noted for HFCS/FD than for control animals in all specimens but the difference was not significant ($P < 0.05$).

Table 3.3: Area under the plasma, and tissue concentrations versus time curve (±SD) for DEA (ng×hr/mL or g) after oral administration of AM 25mg/kg to rats (n=16/group). No significant changes have been found between two groups (using Bailer's method).

Specimen	Group	
	Control	HFCS/FD
Plasma	966 ± 151	811 ± 133
Liver	113581 ± 35824	145905 ± 48223
Heart	47267 ± 11682	40015 ± 6860
Lung	144314 ± 39606	113362 ± 23237

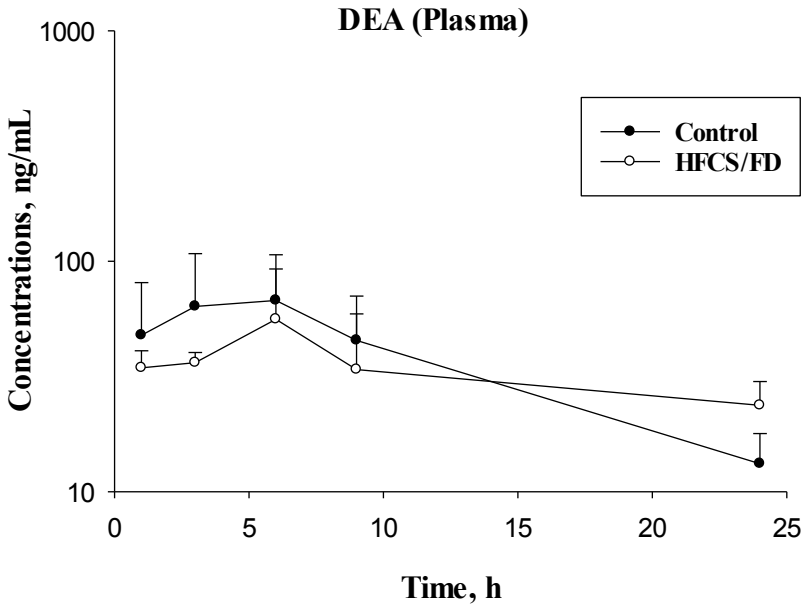


Figure 3.24: Concentration vs. time profiles of DEA in the Plasma following oral administration of AM 25mg/kg. All data are presented as mean + SD (n =16/group). * Denotes a significant difference in DEA concentrations at that time point between control and HFCS/FD groups ($p<0.05$, unpaired t-test).

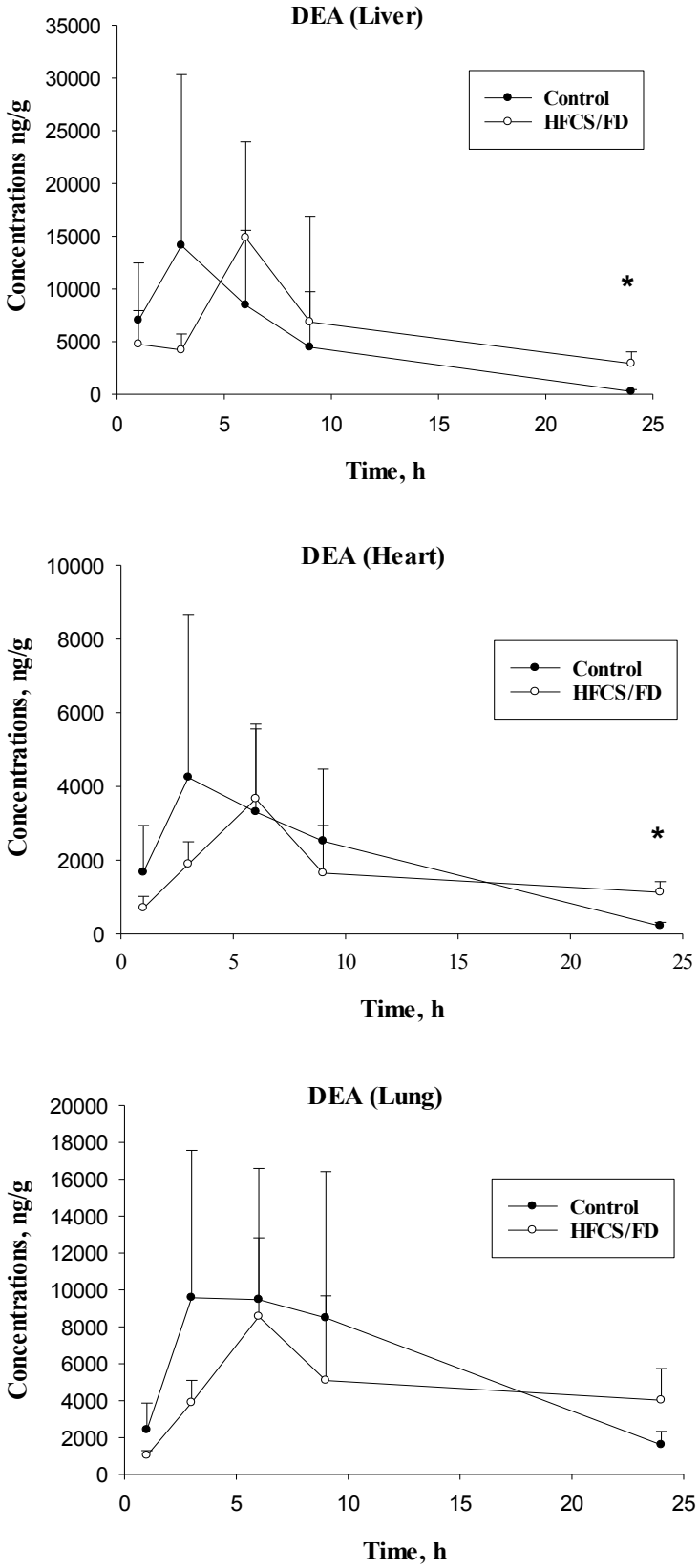


Figure 3.25: Concentration vs. time profiles of DEA in Liver (Top panel), Heart (Middle panel) and Lung (Lower panel) following oral administration of AM 25mg/kg. All data are presented as mean + SD (n =16/group). * Denotes a significant difference in DEA concentrations at that time point between control and HFCS/FD groups ($p<0.05$, unpaired t-test).

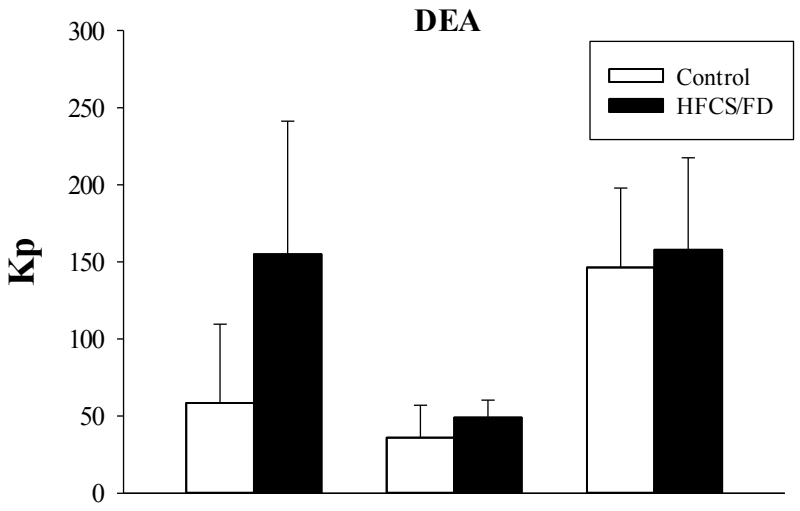


Figure 3.26: Kp vs. time profiles for DEA after oral administration of AM 25mg/kg. The data presents Liver, Heart and Lung to plasma ratios for DEA. All data presented as mean + SD (n = 16/group). No significant difference in Kp of DEA between control and HFCS/FD groups ($p<0.05$, unpaired t-test).

CHAPTER 4: DISCUSSION

4.1. Rat model of obesity

Previous studies have reported that obesity is associated with changes in pharmacokinetics of some drugs, but there were some discrepancies between the findings of those studies (44, 46, 47, 56, 59, 60, 74). Our study was designed to study the effect of obesity on the expression of some CYPs enzymes, transporters and on pharmacokinetics of AM in male rats. We hypothesized that obesity may play a role in down-regulation of number of CYPs enzymes and transporter proteins with consequences on absorption, distribution, or elimination of drugs. To test for this hypothesis, we developed an animal model in which male rats were fed a diet that is similar to commonly consumed fast food or what so called Western-lifestyle. Thus, we considered the widespread use of high fructose corn syrup in the food industry and high consumption of fat containing diet, thereby developing an obesity model in which rats fed ad libitum a high-fructose corn syrup in their drinking water and /or high-fat chow for 14 weeks.

4.2. Validation of the animal model: The effect of high caloric diet on body weight and fat mass

HFCS group end weight was significantly higher compare to that of control (639 vs. 719 g) suggesting that high caloric fructose containing sweeteners can lead to increase the frequency of dietary related obesity (Fig. 3.1). Although the daily caloric consumptions between high calorie groups were close, the high fat diet resulted in a higher body weight in FD (789 g) and HFCS/FD (802 g) groups compared to HFCS group. Thus, high fat diet has more effect on rats' adiposity (Fig. 3.1, 3.4). This was clearly observed in the pre-nephric fat mass (fat mass around the kidney), which was significantly higher in FD

group (44.8 g) compared to control (13.3 g) and HFCS (27.3 g) groups, and higher but not significant compared to HFCS/FD (41.5) group.

4.3. The effect of diet induced obesity on the levels of cholesterol, triglyceride and HDL

We observed some changes in plasma biomarkers including elevated plasma level of cholesterol and triglyceride, which are some characteristic features associated with obesity. However, these elevations were depending on the diet ingested by rats. In case of cholesterol the elevation were significant in FD and HFCS/FD, which seemed to be related to the elevated fat component of the diet (Fig. 3.5). In contrast, triglyceride elevations were significant in HFCS and HFCS/FD apparently due to the HFCS component since FD alone caused no such change (Fig. 3.5). In contrast, HDL was higher only in FD group (Fig. 3.5).

It is known that obesity is associated with HL. It is reported that HL can change the pharmacokinetics of some drugs especially lipophilic drug with high affinity to plasma lipoproteins such as AM (51), halofantrine (109) and amphotericin B (144). HL can decrease the unbound fraction (fu) of a drug in the plasma through increase in the binding of this drug to lipoproteins, which consequently lead to increase in its plasma concentration and decrease its clearance and Vd. Only free drug can be metabolized in liver therefore a decrease in the unbound fraction could lead to decrease in the metabolism of the drug in the liver.

Further, it is reported that HL could also have influence on the expression of metabolizing enzymes and transporters in liver (106). For example, hypertriglyceridemia

has been reported to concur with down regulation of enzymes such as CYP2C11 and ABC transporters such as ABCG5 and ABCG8 (145). In addition, in P407 induced HL rat model there were down regulation in CYP3A1, CYP 3A2 and CYP2C11 in HL compared to normolipidemic rats (104). Our model demonstrated increases in these LP, therefore, it is expected that HL could be one of the reason behind the obesity-associated changes in proteins expression.

4.4. Diet induced obesity leads to hyperinsulinemia

It has been reported that diabetes is associated with some changes in the expression of some CYPs enzymes. In our model, we tried to omit the effect of diabetes on the expression of CYPs and transporters. Our results showed that insulin levels have been increased in all high caloric diet groups; however, the glucose levels were not high enough to consider those animals diabetic (Fig. 3.6). This means that the reason for elevated levels of insulin was to keep down plasma glucose concentrations, which it was successful in doing. The elevated insulin concentrations are consistent with previous finding that hyperinsulinemia is one of pathological feature in obesity (146). We believe that the rats in this study were pre-diabetic, which is characterized by hyperinsulinemia, small elevation in glucose level and higher urination level than control rats.

The reason to exclude diabetes from our study is that diabetes itself can affect the expression level of some transporter proteins and thereby interfere with our results regarding the mechanism behind obesity effect on these transporters. Diabetes has been reported to change the expression of some transporters but its effect showed some inconsistencies. One study involving the streptozotocin (STZ) rat model of diabetes

demonstrated that the hepatic expression of OATP2 was increased leading to increase in liver concentration of pravastatin, however, the expression of MRP2 was decreased leading to decrease in biliary excretion of pravastatin (147). The decrease in MRP2 is in agreement with other findings that the hepatic mRNA expression of *Mdr1* and *Oatp1* were significantly decreased in STZ-induced diabetic rats. Also, study on same rat model showed that diabetes leads to a decrease in tetraethylammonium renal uptake mediated by OCT transporters and this was inhibited by insulin treatment (148). In contrast, another study showed that the expression of the MDR2 was increased using the same model (149). One must consider though that this model of diabetes is really Type 1 diabetes, which is characterized by lack of insulin expression and severe loss in body weight. In terms of type 2 diabetes, an increase the expression of CYP3A enzymes in murine models of type 2 diabetes (150). Also, a decrease in the level of renal OCT2 was observed in type 2 diabetes rat model induced by high fat diet and single dose of STZ (80).

4.5. The effect of diet induced obesity on the levels of some inflammatory mediators

In an experimental setting, inflammation has been found to concur with changes in transporter expression. Hepatic and intestinal P-glycoprotein has been found to decrease in inflammation condition in rats (151). Also, the in vitro study on human hepatocytes revealed that transporters encoded by SLC genes, including OCT1, OAT2 and OATP1B1/1B3/2B1 were decreased when exposed cells to TNFα and IL6 (123). Further, TNFα, IL6, LPS have reported to down regulate some CYPs enzymes including CYP3A4, 2C8, 2C9 and 3B6 (120).

It is reported that increased adiposity associated with obesity can lead to increase number of inflammatory mediators like TNFα, leptin, and others pro-inflammatory cytokines and decrease in adiponectin levels (119). In this study we found that plasma levels of leptin increased significantly in all high caloric-diet groups (Fig. 3.7). We also find significant increase in plasma levels of GIP in FD group only. However, we did not find any significant differences between treated and control groups in the plasma levels of amylin (active), ghrelin, PYY, TNFα or adiponectin (Table 3.1).

Leptin is known to has role in immune response through stimulation of proinflammatory cytokines production (152). The increased in leptin levels in our study did not concur with increase in some measured cytokines. This increase could be attributed to decrease in the sensitivity to leptin (leptin resistant) or due to decrease in the leptin receptors.

Although we did not find any significant changes in cytokines levels, it might be necessary to measure the concentration of these inflammatory mediators in the tissue samples of liver, kidney and intestine. Thus, we can confirm if these cytokines have role in changing the expression levels of the measured enzymes and transporters.

4.6. Diet induced obesity and the changes in ALT and AST levels

Liver damage has been reported to affect the expression of some CYPs enzymes and transporters. The expression of CYP2C11 has been found to decrease in liver cirrhosis (153). Also, it has been reported that CYP2E1 was induced in rat model of non-alcoholic steatohepatitis (154).

ALT and AST are clinically used as biomarker for liver injury (155). During liver injury, the plasma levels of ALT and AST are known to increase (156, 157). In our model, ALT

and AST levels did not change significantly between groups suggesting that the rats did not develop liver damage (Fig. 3.8). Therefore, liver damage has been omitted in our model, and does not have an effect on expression levels of CYPs enzymes or transporters.

4.7. The effect of diet induced obesity on the expression levels of CYP enzymes in the liver

The obesity-related alterations in the expression of CYPs enzymes and transporter proteins have been shown to occur in genetically and diet-induced obese animal models. However, these changes varied from one enzyme to another and from one study to another.

Our findings demonstrated that the CYP3A and 2C enzymes were affected by obesity. *Cyp3a2* mRNA levels were decreased in all high caloric diet groups, which is concur with decreased in protein levels of CYP3A2 (Fig. 3.9). Although the mRNA of *Cyp3a1* and *Cyp2c11* showed non-significant decrease, their protein expressions were significantly decreased in both FD and HFCS/FD groups (Fig. 3.10, 3.11). With CYP3A family and 2C family counts for 50% and 16 % of metabolism in liver, the decrease in activity of these enzymes could affect the metabolism of wide range of endogenous and exogenous compounds. Our results were in line with the previous report where a rodent model of obesity had been shown to down regulate the expressions of CYP3A and CYP2C hepatic drug metabolizing enzymes (61). Further, *Cyp1a1* showed non-significant changes in mRNA levels between all groups with small decrease in protein levels of FD and HFCS/FD and a little increase in HFCS (Fig. 3.12).

Further, there were significant decreases in *Udpgt2b1* in all high caloric diet groups compared to control. However, *Udpgt1a1* showed trend toward decrease in these groups but were not significant (Fig. 3.13).

Our results demonstrated discrepancies between the mRNA levels and protein expression in which sometimes the mRNA level of an enzyme decreases and its protein expression increases or the opposite, and other times both of them decreasing. This is in line with previous observations that mRNA levels and their protein expression can be different (158), which could be related to the problem with stability of specific gene and its rate of translation or protein stability issues (159).

Overall, the discrepancies that have been displayed in literature about the effect of obesity on CYPs enzymes and transporters expression are attributable to: i) the species-specific differences in obesity. The expression of metabolizing enzymes and transporter protein can be different from mice to rats to guinea pigs, which are different from human (56-58). ii) Sex differences. Female model has showed differences in expression of some enzymes and transporters compared to male models (57, 59). This could be attributable to the genetic and hormonal differences between two genders. A previous study reported that male rats are more susceptible to weight gain and symptoms associated with metabolic syndrome than female rats, and that testosterone doses to female rats reduced some of the metabolic differences between two genders (160). Another study revealed that CYP enzyme expression is different in female and male obese mice (67). Further, unlike our study, Ghonium et al. (59) demonstrated in their study that female Sprague–Dawley rats showed significant increases in the CYP3A2 and MDR1 expressions. We found decreases in both enzymes. There were some fundamental differences though

between the 2 studies involving the animals used and the outcomes of weight gain. It was already known prior to their study that female rats are relatively resistant to weight gain in response to feeding a high fat diet. Indeed the female rats fed a high fat diet by Ghonium et al. experienced only a 7% increase body weight compared to control normal diet female rats after 13 weeks of feeding. In contrast, in our study the male rats of same strain of FD group (group that resemble female rats in Ghonium et al. study) were over 25% higher in body weight compared to the control normal diet rats. Thus, the female rats did gain relatively little weight and indeed might not be considered obese albeit overweight (59). iii) Diet induced obesity model versus genetic models of obesity. Obesity induced by genetic modification is targeted for specific genes. High calorie-diets models can have different effects depending on the dietary content. Some studies used high fat diets where up to 60% of its caloric content comes from fat, compared to other studies (45, 59, 62). Furthermore, the duration that those animals being kept on high caloric diet could result in different stages of obesity and thereby different expression of CYPs enzymes and transporter proteins. iv) The differences in degree of obesity between studied individuals can lead to variability in interstudy-outcomes. It should be noted that different stages of obesity are associated with different pathological and physiological conditions. This must be taken in consideration during investigation of obesity on enzymes and transporters activity. v) Diseases associated with obesity. Obesity could be associated with other diseases that known to affect genes expression. For example, diabetes has been reported to increase the expression of CYP3A enzymes in mice models of type 1 and type 2 diabetes (150). Also, another study reported that transporter protein such as MRP2 and MDR1 decreased in STZ-induced diabetes rat model (161). Further, a

decreased in the level of renal OCT2 was observed in type 2 diabetes rat model induced by high fat diet and single dose of STZ (80). Infection has found to affect the hepatic expression of some enzymes, which in most cases lead to decrease or suppress their metabolizing activity (125). Also, inflammation and alteration in cytokines levels have been linked to the change in expression of metabolizing enzymes and transporter proteins. For example, IL-6 and IL-1β were found to have inhibitory effects on the expression and activity of MDR1 in cultured hepatocytes (162). Furthermore, CYP3A4 has reported to be down regulated in inflammatory conditions accompanied by high levels of pro-inflammatory cytokines such as IL-6 (121, 163). Also, CYP could be independently regulated in response to obesity. This has been seen with previous studies on the effects of inflammation and infection on CYP450s levels (120).

4.8. The effect of diet induced obesity on the expression levels of transporter proteins in the liver and Kidneys

Drug metabolizing enzymes are located intracellularly in the endoplasmic reticulum membrane; therefore, in order for drugs to be metabolized they must pass through plasma membrane into intracellular space then to these enzymes. In many cases this process involves specific transport proteins located on the cell membrane. Further, for metabolites to get out of the cell it prerequisites the presence of other membrane transporter protein to mediate their excretion out of the cell.

Our results had showed non-significant changes in the liver mRNA of transporter proteins *Mate1, Mdr1*and *Oct1* between all groups (Fig. 3.14.A, 3.15.A, 3.16.A). In contrast, western-blotting results showed us significant decrease in protein levels of

OCT1 in HFCS/FD (Fig. 3.16.B), and significant decreases in MATE1 in both FD and HFCS/FD groups (Fig. 3.14.B). MDR1 showed slight down regulation in all high caloric diet groups but was not significant (Fig. 3.15.B).

In case of kidney, we found that there was a slight down regulation in *Oct2* mRNA in all high caloric diet groups with significant decrease in OCT2 proteins in both FD and HFCS/FD groups (Fig. 3.18). Also, the OCT1 protein expression was significantly decreased in HFCS/FD group only (Fig. 3.17). In contrast, both MATE1 and MDR1 displayed non-significant changes in both mRNA and protein levels (Fig. 3.19, 3.20).

OCT1 is influx transporter that involves in transportation of many drugs such as antiarrhythmic drug (Quinidine), Hypoglycemic drug (metformin), antimicrobial (Ciprofloxacin), different antineoplastic agent (Paclitaxel, Imatinib, and Sorafenib) and many other drug classes (77). The decrease in OCT1 protein expression level could affect liver uptake of these drugs. Also, this can indirectly influence the rate and extent of drug CL by minimizing the amount of drug that can access the metabolizing enzymes. Our results contradict with previous finding that hepatic OCT1 is up regulated in obese mice fed high fat diet for 19 weeks (78). This contradiction could be due to animal type, diet used to induce obesity or the duration of study.

MDR1 is efflux transporters that mediate the transfer of drugs to extracellular space. Many therapeutic drugs are substrate for MDR1 including daunorubicin, doxorubicin, vinblastine, digoxin, morphine, cyclosporine A and others (164). Our results agreed with previous finding that the mice hepatic expressions of MDR1A were not significantly

affected by obesity (61). In contrast, our results contradict with another study, which demonstrated that using high fat diet in Sprague–Dawley female rats leads to increase in hepatic expression of MDR1 (59), which as explained above that female rats in this study were not obese.

Further, MATE1 is also efflux transporter and mediate transfer of drug such as metformin, cimetidine, cephalexin, cisplatin, procainamide and captopril (81). The down regulation that we find in high fat fed rats can be of great significant in case of using the above-mentioned drugs.

Table 4.1. Summary table for changes observed in plasma biochemical parameters and enzymes/transporters in both liver and kidneys in our model of dietary induced obese rat. Significant decrease (↓), significant increase (↑), non-significant changes (ND), not measured (-).

Sample	Parameter	HFCS		FD		HFCS/FD	
Plasma	Glucose	**ND**		**ND**		↑	
Plasma	Insulin	↑		↑		↑	
Plasma	Cholesterol	**ND**		↑		↑	
Plasma	Triglyceride	↑		**ND**		↑	
Plasma	HDL	**ND**		↑		**ND**	
Plasma	ALT	↓		**ND**		**ND**	
Plasma	AST	**ND**		**ND**		**ND**	
Plasma	Leptin	↑		↑		↑	
Tissue	Enzyme/ Transporter	HFCS		FD		HFCS/FD	
		mRNA	Protein	mRNA	Protein	mRNA	Protein
Liver	CYP3A1	**ND**	**ND**	**ND**	↓	**ND**	↓
Liver	CYP3A2	↓	**ND**	↓	↓	↓	↓
Liver	CYP2C11	**ND**	**ND**	**ND**	↓	**ND**	↓
Liver	CYP1A1	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
Liver	UDPGT1A1	**ND**	-	**ND**	-	**ND**	-
Liver	UDPGT2B1	↓	-	↓	-	↓	-
Liver	OCT1	**ND**	**ND**	**ND**	**ND**	**ND**	↓
Kidney	OCT1	**ND**	**ND**	**ND**	**ND**	**ND**	↓
Kidney	OCT2	**ND**	**ND**	**ND**	↓	**ND**	↓
Liver	MATE1	**ND**	**ND**	**ND**	↓	**ND**	↓
Kidney	MATE1	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
Liver	MDR1	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
Kidney	MDR1	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**

4.9. The effect of diet induced obesity on a single dose biodistribution of AM and its metabolite (DEA)

Due to previous findings that obesity is associated with some changes in CYP and transporters expression, and our finding that demonstrated a decrease in CYPs enzymes in most of treated groups especially HFCS/FD group, it was important to examine the biological effects of these changes on biodistribution of clinically used drug (AM). To our knowledge, we performed the first biodistribution study that has examined AM levels in a rat model of diet-induced obesity.

Our results demonstrated a significant increase in the plasma AUC of AM in obese rats compared with control rats (Table 3.2). This can be explained by high levels of plasma lipoproteins in HFCS/FD group, as suggested by higher plasma cholesterol and triglyceride (Fig. 3.5). AM is a lipophilic drug which extensively bound to plasma proteins especially plasma lipoprotein, therefore, high lipoprotein levels can lead to increase in amount of bound drug, and thereby increase in plasma concentration of AM. The increased in bound drug fraction of AM in HFCS/FD group led to decrease in its tissue distribution, described by reduction in AUC and Kp values of AM in both heart and lung compared to control (Fig. 3.22, 3.23).

Further, the increase in AUC of AM can be related to decrease in the CYP enzyme activity in liver and intestine. It is know that AM can be metabolized by several enzymes in liver and intestine. The major enzymes involved in AM metabolism in rats are CYP2D1, CYP2C11 and CYP3A2 (131). Although we did not measure the expression level of P450 enzymes in intestine, our finding from the liver suggest that the level of

some of these enzymes could be decreased leading to increase in the fraction of drug that reach the systemic circulation.

Kp and AUC of AM were higher in liver of HFCS/FD compare to control group (Fig 3.23). This is can be explained by our finding that the expression level of metabolizing enzymes in liver was decreased. The in vitro incubation of AM with CYP demonstrated that AM can be metabolized by different CYP450 in the rat liver especially 2D1, 2C11, 3A1, and 1A1 (131). From our experiment we found a decrease in CYP3A1, 3A2, and 2C11 (Fig 3.9 - 3.11), which lead to increases in the amount of unmetabolized drug in the liver. Further, as mentioned before HL is concurred with higher plasma LP levels. Theoretically, it is expected that the increases in LP levels lead to decrease in the fu of moderate and low extraction E drugs and thereby lower their Vd. However, the hepatic concentrations of AM (lipophilic drug with moderate hepatic extraction ratio) were actually higher in obese rats. This behavior could possibly be due to the action of hepatic LP receptors, which can mediate transport of LP-bound drugs into liver. These results are in agreement with previous finding that HL is associated with high levels of AM in liver (104). The AUC of DEA was higher in plasma, lung and heart of control compared to the HFCS/FD group (Fig 3.24, 3.25). This is in line with a decrease in metabolism of AM in obese rats. In contrast, the higher value of DEA in the liver of obese rats can be explained by the fact that DEA itself can undergo further metabolism by liver metabolizing enzymes. These enzymes were decreased in obese group as been explained.

4.10. Conclusion

1. Obesity is associated with down regulation of CYP 3A1, 3A2, 2C11 in the liver and transporters MATE1 and OCT1 in liver
2. Obesity is associated with down regulation of OCT1 and OCT2 transporters in the kidney.
3. The degree of down regulation due to obesity is enzyme/transporter dependent. While some of them were highly affected, others showed no change at all.
4. The biodistribution of AM and DEA were changed with obesity.
5. HL could be the main reason for biodistribution changes through increase in the bound fraction of AM in the plasma, or through its effect on the enzymes/transporters expressions. This need to be further explored.
6. Further research is needed to elucidate the mechanism through which obesity affects drug pharmacokinetics.

4.11. Future directions

1. To determine the expression of CYP2D and CYP2E isoforms in liver tissue of obese rats. In human, CYP2D6 is important for metabolism of many drugs such as deprisoquine and codeine. In contrast, CYP2E1 is responsible for metabolism of drugs such as chlorzoxazone, acetaminophen and halothane. Thus, assessment of expression levels of these enzymes could be of great significant for metabolism of these drugs.
2. To determine the expression of OATP transporter proteins that mediates transportation of organic anions compounds across cell membrane. The

importance of this family of transporters comes from its role in excretion of many endogenous and exogenous compounds in the bile.

3. To determine the expression of nuclear receptors such as PXR and CAR in the liver of obese rats. These have very important role in regulation of CYP enzymes.
4. To assess the effect of obesity on transporters function using metformin pharmacokinetics. Because of the important role of transporters in metformin pharmacokinetics, metformin will be used as model drug to assess the functional activity of OCT1, OCT1 and MATE1. Rats will be fed high caloric diet for 14 weeks as described before. Then, rats will have a jugular vein cannula surgically implanted under anesthesia. After that, rats will be given either iv or oral doses of metformin. Thereafter, serial blood samples and urine will be collected for up to 24 h. After 24 h rats will be euthanized under anesthesia and then tissues from liver, kidney and intestine will be collected. All samples will be assayed for metformin using previously published HPLC method.
5. To assess the functional activity of CYP450 enzymes using rat liver microsomes. Liver microsomes from obese and control rats will be incubated with AM. Then, the amount of AM and DEA after incubation will be measured using the same HPLC method used in this study.
6. In our study, we used only single oral dose of amiodarone. Amiodarone has long half-life; therefore, additional study examining the effect of obesity on amiodarone after multiple dosing would be required in order to mimic clinical practice. Also, iv dosing is essential in order to further understand the influence of obesity on amiodarone bioavailability after oral dosing.

7. To see whether changes due to high caloric diet could be reversed by normalization of diet. It is important to explore whether switching to normal diet could results in reversible changes in the expression of drug metabolizing enzymes and transporters. Rats will be fed high caloric diet for 14 weeks as described in this study. Then, there will be an additional 4 weeks in which the rats will be fed a daily restricted diet of 10 g standard rat chow and normal drinking water.

REFERENCES

1. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet. 2005;365(9468):1415-28.
2. Katsiki N, Athyros VG, Karagiannis A, Mikhailidis DP. Metabolic syndrome and non-cardiac vascular diseases: an update from human studies. Curr Pharm Des. 2014;20(31):4944-52.
3. Tzimas P, Petrou A, Laou E, Milionis H, Mikhailidis DP, Papadopoulos G. Impact of metabolic syndrome in surgical patients: should we bother? Br J Anaesth. 2015;115(2):194-202.
4. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. Curr Opin Cardiol. 2006;21(1):1-6.
5. Galassi A, Reynolds K, He J. Metabolic syndrome and risk of cardiovascular disease: a meta-analysis. Am J Med. 2006;119(10):812-9.
6. Bonora E, Kiechl S, Willeit J, Oberhollenzer F, Egger G, Bonadonna RC, et al. Carotid atherosclerosis and coronary heart disease in the metabolic syndrome: prospective data from the Bruneck study. Diabetes Care. 2003;26(4):1251-7.
7. Boden G, Shulman GI. Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and beta-cell dysfunction. Eur J Clin Invest. 2002;32 Suppl 3:14-23.
8. Ginsberg HN. Insulin resistance and cardiovascular disease. J Clin Invest. 2000;106(4):453-8.
9. Organization WH. Obesity and overwieght [web page]. 2015 [updated 01/2015Oct/01/2015].
10. Wright S, Aronne L. Causes of obesity. Abdom Imaging. 2012;37(5):730-2.
11. Brocks DR, Hamdy DA, Ben-Eltriki M, Patel JP, El-Kadi AO. Effect of rat serum lipoproteins on mRNA levels and amiodarone metabolism by cultured primary rat hepatocytes. J Pharm Sci. 2013;102(1):262-70.
12. Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. The disease burden associated with overweight and obesity. JAMA. 1999;282(16):1523-9.
13. Almeda-Valdes P, Aguilar-Olivos N, Uribe M, Mendez-Sanchez N. Common features of the metabolic syndrome and nonalcoholic fatty liver disease. Rev Recent Clin Trials. 2014;9(3):148-58.
14. Flegal KM, Kit BK, Orpana H, Graubard BI. Association of all-cause mortality with overweight and obesity using standard body mass index categories: a systematic review and meta-analysis. JAMA. 2013;309(1):71-82.
15. Flegal KM, Graubard BI, Williamson DF, Gail MH. Excess deaths associated with underweight, overweight, and obesity. JAMA. 2005;293(15):1861-7.
16. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. N Engl J Med. 2003;348(17):1625-38.
17. Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer. 2004;4(8):579-91.
18. Redinger RN. Fat storage and the biology of energy expenditure. Transl Res. 2009;154(2):52-60.

19. Fantuzzi G. Adipose tissue, adipokines, and inflammation. J Allergy Clin Immunol. 2005;115(5):911-9; quiz 20.
20. Das UN. Obesity: genes, brain, gut, and environment. Nutrition. 2010;26(5):459-73.
21. Lutz TA, Woods SC. Overview of animal models of obesity. Curr Protoc Pharmacol. 2012;Chapter 5:Unit5 61.
22. Xu B, Goulding EH, Zang K, Cepoi D, Cone RD, Jones KR, et al. Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. Nat Neurosci. 2003;6(7):736-42.
23. Mandelblat-Cerf Y, Ramesh RN, Burgess CR, Patella P, Yang Z, Lowell BB, et al. Arcuate hypothalamic AgRP and putative POMC neurons show opposite changes in spiking across multiple timescales. Elife. 2015;4.
24. Millington GW. The role of proopiomelanocortin (POMC) neurones in feeding behaviour. Nutr Metab (Lond). 2007;4:18.
25. Muluke M, Gold T, Kiefhaber K, Al-Sahli A, Celenti R, Jiang H, et al. Diet-Induced Obesity and Its Differential Impact on Periodontal Bone Loss. J Dent Res. 2015.
26. Bocarsly ME, Powell ES, Avena NM, Hoebel BG. High-fructose corn syrup causes characteristics of obesity in rats: increased body weight, body fat and triglyceride levels. Pharmacol Biochem Behav. 2010;97(1):101-6.
27. Tetri LH, Basaranoglu M, Brunt EM, Yerian LM, Neuschwander-Tetri BA. Severe NAFLD with hepatic necroinflammatory changes in mice fed trans fats and a high-fructose corn syrup equivalent. Am J Physiol Gastrointest Liver Physiol. 2008;295(5):G987-95.
28. Wadden TA, Volger S, Tsai AG, Sarwer DB, Berkowitz RI, Diewald LK, et al. Managing obesity in primary care practice: an overview with perspective from the POWER-UP study. Int J Obes (Lond). 2013;37 Suppl 1:S3-11.
29. Wadden TA, Neiberg RH, Wing RR, Clark JM, Delahanty LM, Hill JO, et al. Four-year weight losses in the Look AHEAD study: factors associated with long-term success. Obesity (Silver Spring). 2011;19(10):1987-98.
30. Patel D. Pharmacotherapy for the management of obesity. Metabolism. 2015.
31. Sweeting AN, Hocking SL, Markovic TP. Pharmacotherapy for the Treatment of Obesity. Mol Cell Endocrinol. 2015.
32. Aguilar-Olivos NE, Almeda-Valdes P, Aguilar-Salinas CA, Uribe M, Mendez-Sanchez N. The role of bariatric surgery in the management of nonalcoholic fatty liver disease and metabolic syndrome. Metabolism. 2015.
33. Sasaki A, Nitta H, Otsuka K, Umemura A, Baba S, Obuchi T, et al. Bariatric surgery and non-alcoholic Fatty liver disease: current and potential future treatments. Front Endocrinol (Lausanne). 2014;5:164.
34. Buchwald H, Oien DM. Metabolic/bariatric surgery worldwide 2011. Obes Surg. 2013;23(4):427-36.
35. Healy P, Clarke C, Reynolds I, Arumugasamy M, McNamara D. Complications of bariatric surgery - What the general surgeon needs to know. Surgeon. 2015.
36. Hanley MJ, Abernethy DR, Greenblatt DJ. Effect of obesity on the pharmacokinetics of drugs in humans. Clin Pharmacokinet. 2010;49(2):71-87.

37. Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999-2004. JAMA. 2006;295(13):1549-55.
38. Brill MJ, Diepstraten J, van Rongen A, van Kralingen S, van den Anker JN, Knibbe CA. Impact of obesity on drug metabolism and elimination in adults and children. Clin Pharmacokinet. 2012;51(5):277-304.
39. Cheymol G. Effects of obesity on pharmacokinetics implications for drug therapy. Clin Pharmacokinet. 2000;39(3):215-31.
40. Goodman LS, Brunton LL, Chabner B, Knollman BC. Goodman & Gilman's the pharmacological basis of therapeutics: New York : McGraw-Hill, 2011.
12th ed. / editor, Laurence L. Brunton ; associate editors, Bruce A. Chabner, Bjorn C. Knollman; 2011.
41. Bowman SL, Hudson SA, Simpson G, Munro JF, Clements JA. A comparison of the pharmacokinetics of propranolol in obese and normal volunteers. Br J Clin Pharmacol. 1986;21(5):529-32.
42. Cheymol G, Weissenburger J, Poirier JM, Gellee C. The pharmacokinetics of dexfenfluramine in obese and non-obese subjects. Br J Clin Pharmacol. 1995;39(6):684-7.
43. Clauson PG, Linde B. Absorption of rapid-acting insulin in obese and nonobese NIDDM patients. Diabetes Care. 1995;18(7):986-91.
44. Sanderink GJ, Le Liboux A, Jariwala N, Harding N, Ozoux ML, Shukla U, et al. The pharmacokinetics and pharmacodynamics of enoxaparin in obese volunteers. Clin Pharmacol Ther. 2002;72(3):308-18.
45. Sugioka N, Haraya K, Fukushima K, Ito Y, Takada K. Effects of obesity induced by high-fat diet on the pharmacokinetics of nelfinavir, a HIV protease inhibitor, in laboratory rats. Biopharm Drug Dispos. 2009;30(9):532-41.
46. Schwartz AE, Matteo RS, Ornstein E, Young WL, Myers KJ. Pharmacokinetics of sufentanil in obese patients. Anesth Analg. 1991;73(6):790-3.
47. Chung EK, Cheatham SC, Fleming MR, Healy DP, Shea KM, Kays MB. Population pharmacokinetics and pharmacodynamics of piperacillin and tazobactam administered by prolonged infusion in obese and nonobese patients. J Clin Pharmacol. 2015;55(8):899-908.
48. Benedek IH, Fiske WD, 3rd, Griffen WO, Bell RM, Blouin RA, McNamara PJ. Serum alpha 1-acid glycoprotein and the binding of drugs in obesity. Br J Clin Pharmacol. 1983;16(6):751-4.
49. Shayeganpour A, Jun AS, Brocks DR. Pharmacokinetics of Amiodarone in hyperlipidemic and simulated high fat-meal rat models. Biopharm Drug Dispos. 2005;26(6):249-57.
50. Brocks DR, Ala S, Aliabadi HM. The effect of increased lipoprotein levels on the pharmacokinetics of cyclosporine A in the laboratory rat. Biopharm Drug Dispos. 2006;27(1):7-16.
51. Shayeganpour A, Lee SD, Wasan KM, Brocks DR. The influence of hyperlipoproteinemia on in vitro distribution of amiodarone and desethylamiodarone in human and rat plasma. Pharm Res. 2007;24(4):672-8.

52. Imaoka S, Hashizume T, Funae Y. Localization of rat cytochrome P450 in various tissues and comparison of arachidonic acid metabolism by rat P450 with that by human P450 orthologs. Drug Metab Pharmacokinet. 2005;20(6):478-84.
53. Lewis DF. Human cytochromes P450 associated with the phase 1 metabolism of drugs and other xenobiotics: a compilation of substrates and inhibitors of the CYP1, CYP2 and CYP3 families. Curr Med Chem. 2003;10(19):1955-72.
54. Evans WE, Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. Science. 1999;286(5439):487-91.
55. Danielson PB. The cytochrome P450 superfamily: biochemistry, evolution and drug metabolism in humans. Curr Drug Metab. 2002;3(6):561-97.
56. Patoine D, Levac X, Pilote S, Drolet B, Simard C. Decreased CYP3A expression and activity in guinea pig models of diet-induced metabolic syndrome: is fatty liver infiltration involved? Drug Metab Dispos. 2013;41(5):952-7.
57. Cheng Q, Aleksunes LM, Manautou JE, Cherrington NJ, Scheffer GL, Yamasaki H, et al. Drug-metabolizing enzyme and transporter expression in a mouse model of diabetes and obesity. Mol Pharm. 2008;5(1):77-91.
58. Ulvestad M, Skottheim IB, Jakobsen GS, Bremer S, Molden E, Asberg A, et al. Impact of OATP1B1, MDR1, and CYP3A4 expression in liver and intestine on interpatient pharmacokinetic variability of atorvastatin in obese subjects. Clin Pharmacol Ther. 2013;93(3):275-82.
59. Ghoneim RH, Ngo Sock ET, Lavoie JM, Piquette-Miller M. Effect of a high-fat diet on the hepatic expression of nuclear receptors and their target genes: relevance to drug disposition. Br J Nutr. 2015;113(3):507-16.
60. Abernethy DR, Greenblatt DJ, Divoll M, Smith RB, Shader RI. The influence of obesity on the pharmacokinetics of oral alprazolam and triazolam. Clin Pharmacokinet. 1984;9(2):177-83.
61. Yoshinari K, Takagi S, Yoshimasa T, Sugatani J, Miwa M. Hepatic CYP3A expression is attenuated in obese mice fed a high-fat diet. Pharm Res. 2006;23(6):1188-200.
62. Khemawoot P, Yokogawa K, Shimada T, Miyamoto K. Obesity-induced increase of CYP2E1 activity and its effect on disposition kinetics of chlorzoxazone in Zucker rats. Biochem Pharmacol. 2007;73(1):155-62.
63. Lucas D, Farez C, Bardou LG, Vaisse J, Attali JR, Valensi P. Cytochrome P450 2E1 activity in diabetic and obese patients as assessed by chlorzoxazone hydroxylation. Fundam Clin Pharmacol. 1998;12(5):553-8.
64. Frink EJ, Jr., Malan TP, Jr., Brown EA, Morgan S, Brown BR, Jr. Plasma inorganic fluoride levels with sevoflurane anesthesia in morbidly obese and nonobese patients. Anesth Analg. 1993;76(6):1333-7.
65. Bentley JB, Vaughan RW, Miller MS, Calkins JM, Gandolfi AJ. Serum inorganic fluoride levels in obese patients during and after enflurane anesthesia. Anesth Analg. 1979;58(5):409-12.
66. Bentley JB, Vaughan RW, Gandolfi AJ, Cork RC. Halothane biotransformation in obese and nonobese patients. Anesthesiology. 1982;57(2):94-7.
67. Roe AL, Howard G, Blouin R, Snawder JE. Characterization of cytochrome P450 and glutathione S-transferase activity and expression in male and female ob/ob mice. Int J Obes Relat Metab Disord. 1999;23(1):48-53.

68. Abernethy DR, Divoll M, Greenblatt DJ, Ameer B. Obesity, sex, and acetaminophen disposition. Clin Pharmacol Ther. 1982;31(6):783-90.
69. Kim MS, Wang S, Shen Z, Kochansky CJ, Strauss JR, Franklin RB, et al. Differences in the pharmacokinetics of peroxisome proliferator-activated receptor agonists in genetically obese Zucker and sprague-dawley rats: implications of decreased glucuronidation in obese Zucker rats. Drug Metab Dispos. 2004;32(9):909-14.
70. Chaudhary IP, Tuntaterdtum S, McNamara PJ, Robertson LW, Blouin RA. Effect of genetic obesity and phenobarbital treatment on the hepatic conjugation pathways. J Pharmacol Exp Ther. 1993;265(3):1333-8.
71. Henegar JR, Bigler SA, Henegar LK, Tyagi SC, Hall JE. Functional and structural changes in the kidney in the early stages of obesity. J Am Soc Nephrol. 2001;12(6):1211-7.
72. Janmahasatian S, Duffull SB, Chagnac A, Kirkpatrick CM, Green B. Lean body mass normalizes the effect of obesity on renal function. Br J Clin Pharmacol. 2008;65(6):964-5.
73. Christoff PB, Conti DR, Naylor C, Jusko WJ. Procainamide disposition in obesity. Drug Intell Clin Pharm. 1983;17(7-8):516-22.
74. Allard S, Kinzig M, Boivin G, Sorgel F, LeBel M. Intravenous ciprofloxacin disposition in obesity. Clin Pharmacol Ther. 1993;54(4):368-73.
75. Fromm MF. Drug transporters. [electronic resource]: Heidelberg ; New York : Springer, c2011.; 2011.
76. Lin L, Yee SW, Kim RB, Giacomini KM. SLC transporters as therapeutic targets: emerging opportunities. Nat Rev Drug Discov. 2015;14(8):543-60.
77. Lozano E, Herraez E, Briz O, Robledo VS, Hernandez-Iglesias J, Gonzalez-Hernandez A, et al. Role of the plasma membrane transporter of organic cations OCT1 and its genetic variants in modern liver pharmacology. Biomed Res Int. 2013;2013:692071.
78. Jang EH, Kim HK, Park CS, Kang JH. Increased expression of hepatic organic cation transporter 1 and hepatic distribution of metformin in high-fat diet-induced obese mice. Drug Metab Pharmacokinet. 2010;25(4):392-7.
79. Moreno-Navarrete JM, Ortega FJ, Rodriguez-Hermosa JI, Sabater M, Pardo G, Ricart W, et al. OCT1 Expression in adipocytes could contribute to increased metformin action in obese subjects. Diabetes. 2011;60(1):168-76.
80. Nowicki MT, Aleksunes LM, Sawant SP, Dnyanmote AV, Mehendale HM, Manautou JE. Renal and hepatic transporter expression in type 2 diabetic rats. Drug Metab Lett. 2008;2(1):11-7.
81. Staud F, Cerveny L, Ahmadimoghaddam D, Ceckova M. Multidrug and toxin extrusion proteins (MATE/SLC47); role in pharmacokinetics. Int J Biochem Cell Biol. 2013;45(9):2007-11.
82. Yonezawa A, Inui K. Importance of the multidrug and toxin extrusion MATE/SLC47A family to pharmacokinetics, pharmacodynamics/toxicodynamics and pharmacogenomics. Br J Pharmacol. 2011;164(7):1817-25.
83. Dean M, Rzhetsky A, Allikmets R. The human ATP-binding cassette (ABC) transporter superfamily. Genome Res. 2001;11(7):1156-66.

84. Garcia-Carrasco M, Mendoza-Pinto C, Macias Diaz S, Vera-Recabarren M, Vazquez de Lara L, Mendez Martinez S, et al. P-glycoprotein in autoimmune rheumatic diseases. Autoimmun Rev. 2015;14(7):594-600.
85. Nawa A, Fujita-Hamabe W, Tokuyama S. Altered intestinal P-glycoprotein expression levels in a monosodium glutamate-induced obese mouse model. Life Sci. 2011;89(23-24):834-8.
86. Wen J, Luo J, Huang W, Tang J, Zhou H, Zhang W. The Pharmacological and Physiological Role of Multidrug-Resistant Protein 4. J Pharmacol Exp Ther. 2015;354(3):358-75.
87. Brocks DR, Ben-Eltriki M, Gabr RQ, Padwal RS. The effects of gastric bypass surgery on drug absorption and pharmacokinetics. Expert Opin Drug Metab Toxicol. 2012;8(12):1505-19.
88. Padwal RS, Gabr RQ, Sharma AM, Langkaas LA, Birch DW, Karmali S, et al. Effect of gastric bypass surgery on the absorption and bioavailability of metformin. Diabetes Care. 2011;34(6):1295-300.
89. Andujar-Plata P, Pi-Sunyer X, Laferrere B. Metformin effects revisited. Diabetes Res Clin Pract. 2012;95(1):1-9.
90. Proctor WR, Bourdet DL, Thakker DR. Mechanisms underlying saturable intestinal absorption of metformin. Drug Metab Dispos. 2008;36(8):1650-8.
91. di Masi A, De Marinis E, Ascenzi P, Marino M. Nuclear receptors CAR and PXR: Molecular, functional, and biomedical aspects. Mol Aspects Med. 2009;30(5):297-343.
92. Ihunnah CA, Jiang M, Xie W. Nuclear receptor PXR, transcriptional circuits and metabolic relevance. Biochim Biophys Acta. 2011;1812(8):956-63.
93. Litwa E, Rzemieniec J, Wnuk A, Lason W, Krzeptowski W, Kajta M. RXRalpha, PXR and CAR xenobiotic receptors mediate the apoptotic and neurotoxic actions of nonylphenol in mouse hippocampal cells. J Steroid Biochem Mol Biol. 2016;156:43-52.
94. Banerjee M, Robbins D, Chen T. Modulation of xenobiotic receptors by steroids. Molecules. 2013;18(7):7389-406.
95. Mo L, He J. Nuclear hormone receptors PXR and CAR and metabolic diseases. Horm Mol Biol Clin Investig. 2014;19(2):129-40.
96. Huang W, Zhang J, Chua SS, Qatanani M, Han Y, Granata R, et al. Induction of bilirubin clearance by the constitutive androstane receptor (CAR). Proc Natl Acad Sci U S A. 2003;100(7):4156-61.
97. Gao J, He J, Zhai Y, Wada T, Xie W. The constitutive androstane receptor is an anti-obesity nuclear receptor that improves insulin sensitivity. J Biol Chem. 2009;284(38):25984-92.
98. Zhou X, Zhang W, Liu X, Zhang W, Li Y. Interrelationship between diabetes and periodontitis: role of hyperlipidemia. Arch Oral Biol. 2015;60(4):667-74.
99. Sawabe M, Tanaka N, Nakahara K, Hamamatsu A, Chida K, Arai T, et al. High lipoprotein(a) level promotes both coronary atherosclerosis and myocardial infarction: a path analysis using a large number of autopsy cases. Heart. 2009;95(24):1997-2002.
100. Antonios N, Angiolillo DJ, Silliman S. Hypertriglyceridemia and ischemic stroke. Eur Neurol. 2008;60(6):269-78.
101. Montague CT, O'Rahilly S. The perils of portliness: causes and consequences of visceral adiposity. Diabetes. 2000;49(6):883-8.

102. Wasan KM, Brocks DR, Lee SD, Sachs-Barrable K, Thornton SJ. Impact of lipoproteins on the biological activity and disposition of hydrophobic drugs: implications for drug discovery. Nat Rev Drug Discov. 2008;7(1):84-99.
103. Olson RE. Discovery of the lipoproteins, their role in fat transport and their significance as risk factors. J Nutr. 1998;128(2 Suppl):439S-43S.
104. Shayeganpour A, Korashy H, Patel JP, El-Kadi AO, Brocks DR. The impact of experimental hyperlipidemia on the distribution and metabolism of amiodarone in rat. Int J Pharm. 2008;361(1-2):78-86.
105. Patel JP, Hamdy DA, El-Kadi AO, Brocks DR. Effect of serum lipoproteins on stereoselective halofantrine metabolism by rat hepatocytes. Chirality. 2012;24(7):558-65.
106. Patel JP, Brocks DR. The effect of oral lipids and circulating lipoproteins on the metabolism of drugs. Expert Opin Drug Metab Toxicol. 2009;5(11):1385-98.
107. Hussain MM, Strickland DK, Bakillah A. The mammalian low-density lipoprotein receptor family. Annu Rev Nutr. 1999;19:141-72.
108. Takahashi S, Sakai J, Fujino T, Hattori H, Zenimaru Y, Suzuki J, et al. The very low-density lipoprotein (VLDL) receptor: characterization and functions as a peripheral lipoprotein receptor. J Atheroscler Thromb. 2004;11(4):200-8.
109. Patel JP, Fleischer JG, Wasan KM, Brocks DR. The effect of experimental hyperlipidemia on the stereoselective tissue distribution, lipoprotein association and microsomal metabolism of (+/-)-halofantrine. J Pharm Sci. 2009;98(7):2516-28.
110. Aliabadi HM, Spencer TJ, Mahdipoor P, Lavasanifar A, Brocks DR. Insights into the effects of hyperlipoproteinemia on cyclosporine A biodistribution and relationship to renal function. AAPS J. 2006;8(4):E672-81.
111. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. J Clin Invest. 2006;116(7):1793-801.
112. Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. Annu Rev Immunol. 2011;29:415-45.
113. Li MD. Leptin and beyond: an odyssey to the central control of body weight. Yale J Biol Med. 2011;84(1):1-7.
114. Hall JE, do Carmo JM, da Silva AA, Wang Z, Hall ME. Obesity-induced hypertension: interaction of neurohumoral and renal mechanisms. Circ Res. 2015;116(6):991-1006.
115. Lihn AS, Bruun JM, He G, Pedersen SB, Jensen PF, Richelsen B. Lower expression of adiponectin mRNA in visceral adipose tissue in lean and obese subjects. Mol Cell Endocrinol. 2004;219(1-2):9-15.
116. Wang C. Obesity, inflammation, and lung injury (OILI): the good. Mediators Inflamm. 2014;2014:978463.
117. Aguilar-Valles A, Inoue W, Rummel C, Luheshi GN. Obesity, adipokines and neuroinflammation. Neuropharmacology. 2015;96(Pt A):124-34.
118. Hotamisligil GS, Murray DL, Choy LN, Spiegelman BM. Tumor necrosis factor alpha inhibits signaling from the insulin receptor. Proc Natl Acad Sci U S A. 1994;91(11):4854-8.
119. Berg AH, Scherer PE. Adipose tissue, inflammation, and cardiovascular disease. Circ Res. 2005;96(9):939-49.

120. Aitken AE, Morgan ET. Gene-specific effects of inflammatory cytokines on cytochrome P450 2C, 2B6 and 3A4 mRNA levels in human hepatocytes. Drug Metab Dispos. 2007;35(9):1687-93.
121. Mimura H, Kobayashi K, Xu L, Hashimoto M, Ejiri Y, Hosoda M, et al. Effects of cytokines on CYP3A4 expression and reversal of the effects by anti-cytokine agents in the three-dimensionally cultured human hepatoma cell line FLC-4. Drug Metab Pharmacokinet. 2015;30(1):105-10.
122. Le Vee M, Gripon P, Stieger B, Fardel O. Down-regulation of organic anion transporter expression in human hepatocytes exposed to the proinflammatory cytokine interleukin 1beta. Drug Metab Dispos. 2008;36(2):217-22.
123. Le Vee M, Lecureur V, Stieger B, Fardel O. Regulation of drug transporter expression in human hepatocytes exposed to the proinflammatory cytokines tumor necrosis factor-alpha or interleukin-6. Drug Metab Dispos. 2009;37(3):685-93.
124. Harvey RD, Morgan ET. Cancer, inflammation, and therapy: effects on cytochrome p450-mediated drug metabolism and implications for novel immunotherapeutic agents. Clin Pharmacol Ther. 2014;96(4):449-57.
125. Morgan ET. Regulation of cytochromes P450 during inflammation and infection. Drug Metab Rev. 1997;29(4):1129-88.
126. Morgan ET, Goralski KB, Piquette-Miller M, Renton KW, Robertson GR, Chaluvadi MR, et al. Regulation of drug-metabolizing enzymes and transporters in infection, inflammation, and cancer. Drug Metab Dispos. 2008;36(2):205-16.
127. Podrid PJ. Amiodarone: reevaluation of an old drug. Ann Intern Med. 1995;122(9):689-700.
128. Kowey PR, Marinchak RA, Rials SJ, Bharucha D. Pharmacologic and pharmacokinetic profile of class III antiarrhythmic drugs. Am J Cardiol. 1997;80(8A):16G-23G.
129. Riva E, Gerna M, Neyroz P, Urso R, Bartosek I, Guaitani A. Pharmacokinetics of amiodarone in rats. J Cardiovasc Pharmacol. 1982;4(2):270-5.
130. Santangeli P, Di Biase L, Burkhardt JD, Bai R, Mohanty P, Pump A, et al. Examining the safety of amiodarone. Expert Opin Drug Saf. 2012;11(2):191-214.
131. Elsherbiny ME, El-Kadi AO, Brocks DR. The metabolism of amiodarone by various CYP isoenzymes of human and rat, and the inhibitory influence of ketoconazole. J Pharm Pharm Sci. 2008;11(1):147-59.
132. Rafeiro E, Leeder RG, Daniels JM, Brien JF, Massey TE. In vitro hepatic, renal, and pulmonary N-dealkylation of amiodarone. Biochem Pharmacol. 1990;39(10):1627-9.
133. Soyama A, Hanioka N, Saito Y, Murayama N, Ando M, Ozawa S, et al. Amiodarone N-deethylation by CYP2C8 and its variants, CYP2C8*3 and CYP2C8 P404A. Pharmacol Toxicol. 2002;91(4):174-8.
134. Camus P, Martin WJ, 2nd, Rosenow EC, 3rd. Amiodarone pulmonary toxicity. Clin Chest Med. 2004;25(1):65-75.
135. Jaworski K, Walecka I, Rudnicka L, Gnatowski M, Kosior DA. Cutaneous adverse reactions of amiodarone. Med Sci Monit. 2014;20:2369-72.
136. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25(4):402-8.

137. Amara IE, Elshenawy OH, Abdelrady M, El-Kadi AO. Acute mercury toxicity modulates cytochrome P450, soluble epoxide hydrolase and their associated arachidonic acid metabolites in C57Bl/6 mouse heart. Toxicol Lett. 2014;226(1):53-62.
138. Anger GJ, Cressman AM, Piquette-Miller M. Expression of ABC Efflux transporters in placenta from women with insulin-managed diabetes. PLoS One. 2012;7(4):e35027.
139. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265-75.
140. Shayeganpour A, Somayaji V, Brocks DR. A liquid chromatography-mass spectrometry assay method for simultaneous determination of amiodarone and desethylamiodarone in rat specimens. Biomed Chromatogr. 2007;21(3):284-90.
141. Shayeganpour A, Brocks D. Joint assay of desethylamiodarone and amiodarone in rat plasma by high performance liquid chromatography. Journal of Pharmacy & Pharmaceutical Sciences. 2003;6:160.
142. Jun AS, Brocks DR. High-performance liquid chromatography assay of amiodarone in rat plasma. J Pharm Pharm Sci. 2001;4(3):263-8.
143. Bailer AJ. Testing for the equality of area under the curves when using destructive measurement techniques. J Pharmacokinet Biopharm. 1988;16(3):303-9.
144. Wasan KM, Brazeau GA, Keyhani A, Hayman AC, Lopez-Berestein G. Roles of liposome composition and temperature in distribution of amphotericin B in serum lipoproteins. Antimicrob Agents Chemother. 1993;37(2):246-50.
145. Orolin J, Vecera R, Markova I, Zacharova A, Anzenbacher P. Differences in hepatic expression of genes involved in lipid homeostasis between hereditary hypertriglyceridemic rats and healthy Wistar rats and in their response to dietary cholesterol. Food Chem Toxicol. 2009;47(10):2624-30.
146. Modan M, Halkin H, Almog S, Lusky A, Eshkol A, Shefi M, et al. Hyperinsulinemia. A link between hypertension obesity and glucose intolerance. J Clin Invest. 1985;75(3):809-17.
147. Hasegawa Y, Kishimoto S, Shibatani N, Inotsume N, Takeuchi Y, Fukushima S. The disposition of pravastatin in a rat model of streptozotocin-induced diabetes and organic anion transporting polypeptide 2 and multidrug resistance-associated protein 2 expression in the liver. Biol Pharm Bull. 2010;33(1):153-6.
148. Grover B, Auberger C, Sarangarajan R, Cacini W. Functional impairment of renal organic cation transport in experimental diabetes. Pharmacol Toxicol. 2002;90(4):181-6.
149. van Waarde WM, Verkade HJ, Wolters H, Havinga R, Baller J, Bloks V, et al. Differential effects of streptozotocin-induced diabetes on expression of hepatic ABC-transporters in rats. Gastroenterology. 2002;122(7):1842-52.
150. Patoine D, Petit M, Pilote S, Picard F, Drolet B, Simard C. Modulation of CYP3a expression and activity in mice models of type 1 and type 2 diabetes. Pharmacol Res Perspect. 2014;2(6):e00082.
151. Kalitsky-Szirtes J, Shayeganpour A, Brocks DR, Piquette-Miller M. Suppression of drug-metabolizing enzymes and efflux transporters in the intestine of endotoxin-treated rats. Drug Metab Dispos. 2004;32(1):20-7.
152. Shi D, Liu YY, Li W, Zhang X, Sun XJ, Xu L, et al. Association between plasma leptin level and systemic inflammatory markers in patients with aggressive periodontitis. Chin Med J (Engl). 2015;128(4):528-32.

153. Murray M, Cantrill E, Mehta I, Farrell GC. Impaired expression of microsomal cytochrome P450 2C11 in choline-deficient rat liver during the development of cirrhosis. J Pharmacol Exp Ther. 1992;261(1):373-80.
154. Weltman MD, Farrell GC, Liddle C. Increased hepatocyte CYP2E1 expression in a rat nutritional model of hepatic steatosis with inflammation. Gastroenterology. 1996;111(6):1645-53.
155. Lin MS, Lin HS, Chung CM, Lin YS, Chen MY, Chen PH, et al. Serum aminotransferase ratio is independently correlated with hepatosteatosis in patients with HCV: a cross-sectional observational study. BMJ Open. 2015;5(9):e008797.
156. Du SY, Zhang YL, Bai RX, Ai ZL, Xie BS, Yang HY. Lutein prevents alcohol-induced liver disease in rats by modulating oxidative stress and inflammation. Int J Clin Exp Med. 2015;8(6):8785-93.
157. Li SQ, Wan XD, Zhu S, Han HM, Xu ZS, Lu HJ. Establishment of a new animal model of azithromycin-induced liver injury and study the molecular pathological change during the process. Hum Exp Toxicol. 2015.
158. Guo Y, Xiao P, Lei S, Deng F, Xiao GG, Liu Y, et al. How is mRNA expression predictive for protein expression? A correlation study on human circulating monocytes. Acta Biochim Biophys Sin (Shanghai). 2008;40(5):426-36.
159. Maayah ZH, Elshenawy OH, Althurwi HN, Abdelhamid G, El-Kadi AO. Human fetal ventricular cardiomyocyte, RL-14 cell line, is a promising model to study drug metabolizing enzymes and their associated arachidonic acid metabolites. J Pharmacol Toxicol Methods. 2015;71:33-41.
160. Kamari Y, Peleg E, Leibowitz A, Grossman E. Blunted blood pressure response and elevated plasma adiponectin levels in female Sprague Dawley rats. Am J Hypertens. 2012;25(5):612-9.
161. Anger GJ, Magomedova L, Piquette-Miller M. Impact of acute streptozotocin-induced diabetes on ABC transporter expression in rats. Chem Biodivers. 2009;6(11):1943-59.
162. Sukhai M, Yong A, Pak A, Piquette-Miller M. Decreased expression of P-glycoprotein in interleukin-1beta and interleukin-6 treated rat hepatocytes. Inflamm Res. 2001;50(7):362-70.
163. Schmitt C, Kuhn B, Zhang X, Kivitz AJ, Grange S. Disease-drug-drug interaction involving tocilizumab and simvastatin in patients with rheumatoid arthritis. Clin Pharmacol Ther. 2011;89(5):735-40.
164. Funk C. The role of hepatic transporters in drug elimination. Expert Opin Drug Metab Toxicol. 2008;4(4):363-79.