The Impact of Obesity on Proteins Involved in Drug Metabolism

by

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Abstract:

The study of obesity has increased in the recent years, as it is considered a major public health concern. In addition, obesity is often associated with several comorbidities such as diabetes mellitus and cardiovascular diseases which require pharmacological intervention. Although several studies have investigated the impact of obesity on drug pharmacokinetics and the expression of drug metabolizing enzymes and transporters, few have examined its effect on the functional activity and the efficiency of drug metabolizing enzymes.

In this thesis, we investigated the impact of obesity on phase I drug metabolizing enzymes (CYP450) functional activity using amiodarone as a test substrate and bisphenol A as a test substrate for phase II drug metabolizing enzymes (UGTs). These compounds were incubated with microsomes of liver and intestine of four groups of rats which were fed for 14 weeks either normal rodent food and water (control), normal rodent chow and high fructose-corn syrup water (HFCS), 45% high fat (HFD) diet and water, or 45% HFD and HFCS. Moreover, both compounds were also exposed to microsomes of lean and obese JCR rats, a model of genetic obesity.

We found significant decrease in the hepatic intrinsic clearance of amiodarone in all groups fed on high caloric diet compared to control with no significant changes in intestinal intrinsic clearance of the drug between the groups. Regarding the functional activity of phase II drug metabolizing enzymes, we have found that the bisphenol A intrinsic clearance in the liver of all obese groups was increased compared to control. In addition, an increase in the intrinsic clearance of the compound was noticed in the intestinal microsomes of HFD and HFCS fed groups compared to control. With

respect to the genetic obesity model, we found a decrease in the hepatic clearance of amiodarone but not with bisphenol A between lean and obese groups.

Our findings indicate that high caloric diet-induced obesity can change the functional activity of drug metabolizing enzymes. This is consistent to literature data showing decreased expression of some of phase I metabolizing enzymes and increased expression of phase II metabolizing enzymes. Hence, obesity can potentially impact the pharmacokinetics of drugs as we reported the changes in efficiency of drug metabolizing enzymes and consequently, this will eventually impact the drug pharmacodynamics and individual response to drugs.

Preface

This thesis is an original work by Marwa Salem Mohammed Al-Agili. The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, Project Name "The impact of obesity on proteins involved in drug metabolism", No. AUP227

This work is dedicated to

My lovely parents, Salem and Sabria, who always have been here with me with their love, supports, and inspiration.

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List of abbreviation and symbols:

°C	Degree Celsius
ABC	Adenosine triphosphate binding cassette
AgRP	Agouti related protein
AM	Amiodarone
ANOVA	Analysis of variance
AUC	Area under the curve
BBB	Blood brain barrier
BCRP	Breast cancer resistance protein
BMI	Body mass index
BPA	Bisphenol A
BPA-G	Bisphenol A glucuronide
BSA	Body surface area
BSEP	Bile salt export pump
CAR	Constitutive androstane receptor
Cl	Clearance
Cmax	Maximum plasma drug concentration
CMS	Cardiac metabolic syndrome
CNS	Central nervous system
CRP	C-reactive protein
CYPs	Cytochrome P450
DEA	Desethylamiodarone
EC_{50}	Half of the maximal effect concentration
ERs	Estrogen receptors
FDA	Food and drug administration
fu	Unbound fraction
FXR	Farnesoid X receptor
GFR	Glomerular filtration rate
GIP	Gastric inhibitory polypeptide
GLP-1	Glucagon-like peptide-1 (active)
H2SO4	Sulfuric acid
HCl	Hydrochloric acid
HDL	High-density lipoprotein
HFCS	High fructose corn syrup(Group or liquid used)
HFD	Group provided high fat diet 45% kcal of fat and normal water
HFD/HFCS	Group provided high fat diet (45% kcal fat) with 13%w/v HFCS
HPLC	High performance liquid chromatography
HL	Hyperlipidemia
IBW	Ideal bodyweight
IFN	Interferon γ
IL	Interleukin
ip	Intraperitoneal
IS	Internal standard
iv	Intravenous
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
LPS	Lipopolysaccharide
m2	Square meters
MATE	Multidrug and toxin extrusion
MC	Melanocortin
MCP-1	Monocyte chemoattractant protein 1
MDR1	Multidrug resistant gene 1
mg	Milligram
MIF	Macrophage migration inhibitory factor
min	Minutes
mL	Milli liter
mM	Milli molar
mRNA	Messenger RNA
MRP	Multidrug resistance protein
Na_2CO_3	Sodium carbonate
ng	Nano gram
NHR	Nuclear hormone receptors
OATPs	Organic anion transporting polypeptides
OCTs	Organic cation transporters
PAI-1	Plasminogen activator inhibitor
PBPK	Physiologically based pharmacokinetic
Pgp	Permeability glycoprotein
PD	Pharmacodynamics
PK	Pharmacokinetics
POMC	Proopiomelanocortin
rpm	Round per minute
RYGB	Roux-en-Y gastric bypass
SAA	Serum amyloid A
SD	Standard deviation
SLC	Solute carrier
t ½	Terminal elimination phase half-life
TBW	Total bodyweight
TG	Triglycerides
TGF	Transforming growth factor-β
Tmax	Time to reach Cmax
TNFα	Tumor necrosis factor alpha
UGTs	UDP-glucuronosyltransferases
UV	Ultra-violet
Vd	Volume of distribution
WHO	World Health Organization
α	Level of significance
αMSH	α -melanocyte-stimulating hormone
μg	Microgram
μL	Microliter

Chapter1: Introduction

1.1 Obesity:

Obesity can be defined as an abnormal adipose tissue accumulation throughout the body due to imbalance between energy intake and energy use [1]. In addition, the World Health Organization (WHO) classify people based on their body mass index (BMI) which is a measure of expressing body weight relative to the height into obese if their BMI is greater than or equal to 30 kg/m^2 (table1) [2].

Table 1: **WHO (BMI)-based classifications.**

BMI (kg/m^2) Class	**Class**
<18.5	Underweight
≥18.5 and <25.0	Normal weight
≥25.0 and <30.0	Overweight
≥30.0	Obese
≥30.0 and <35.0	Obese class I (moderate obesity)
≥35.0 and <40.0	Obese class II (severe obesity)
≥40.0	Obese class III (morbid obesity)

The prevalence of obesity has increased dramatically worldwide in the recent years. According to WHO in 2014, the estimation of obese and overweight individuals aged 18 years and over was around 13% and 39% of the adult world population respectively. Thus, nearly 2 billion are overweight and more than half a billion are obese in the world [3]. In Canada specifically, around 5.3 million adults reported as

obese and the rate of obesity among men and women were 21.8% and 18.7% respectively in 2014 [4].

Obesity is a major public health problem because it may predispose people to several disease conditions such as type 2 diabetes and insulin resistance, hyperlipidemia, atherosclerosis, hypertension, osteoarthritis, respiratory diseases, and eventually reducing the quality of life. Furthermore, according to several studies, obesity is associated with increased cancer risk such as oesophageal, pancreatic, hepatic, ovarian, and colorectal cancers. In addition, the BMI has been shown to be correlated with the survival rate reduction in patients having liver, gallbladder, pancreas, kidney, and colon cancers [5, 6].

Studies reported that obese individuals have higher mortality rates compared to non-obese individuals due to associated cardiovascular diseases [7, 8].

There are multiple factors that can cause obesity. The increase in the intake of high caloric diet that is rich in saturated and trans fat and carbohydrates accompanied with the decrease in the physical activity (energy intake and output), is considered to be the main factor causing obesity. Furthermore, the resulting changes in the modern life style have a direct effect on the physical activity. Examples for that, the more use of public transportation and leisure activities such as television watching and playing video games instead of performing some types of sports or exercise [9]. Other factors such as polymorphisms in the genes that control metabolism and appetite, medications such as psychotropic drugs, diabetic therapy, steroid hormones and contraceptives, can be attributed to cause obesity [10].

1.2 Physiological changes associated with obesity:

There are some physiological and pathophysiological changes occur with obesity that can affect drug disposition. Morbid obesity is also associated with an increase in lean body weight (LBW; body weight without adipose tissues) representing 20-40% of the excess weight in addition to the increase in percentage of fat mass per kilogram of total body weight. Hence, the supply demand increases for this excess of body mass for the required oxygen, nutrients, blood volume, capillary flow, and cardiac output in obese subjects [11].

The increased blood volume and cardiac output can lead to hypertension, left and right ventricular hypertrophy, and conduction disorder and thereby increasing the risk of sudden cardiac death. Moreover, many studies reported the alteration in pulmonary function due to the reduced lung volume with high incidence of obstructive sleep apnea syndrome [11].

Due to the accumulation of fat in the liver, this can cause fatty liver infiltration and non-alcoholic steatohepatitis that can cause changes in the liver function in morbidly obese patients as many studies reported changes in the expression and the function of liver metabolising enzymes [11].

With respect to the renal function, although some studies found no alteration in the renal function, others reported an increase in glomerular filtration rate ended with end-stage renal disease in genetically obese Zucker rats. Furthermore, researchers pointed out the presence of focal glomerular sclerosis and diabetic nephropathy in morbidly obese patients having proteinuria. Therefore, there is an increased indication to use LBW in the Cockcroft-Gault formula to estimate the correct creatinine clearance for

obese patients [11]. Regarding the gastrointestinal tract function in obesity, obese patients are subjected to high splanchnic blood flow, accelerated gastric emptying, and high permeability of gut wall.

In summary, due to some pathophysiological changes presented in obese patients, individuals administering medications for obesity comorbidities might have altered pharmacokinetics and pharmacodynamics of drugs. Thus, adjustment of the dosage regimen might be required especially for those drugs that have a narrow therapeutics index [12].

1.3 Animal Models of Obesity:

Diverse animal models of obesity have been used to study pharmacokinetics. Some of them are based on exposing the animals to some factors that produce obesity such as supplementation with diets containing high calories and others based on mutation or manipulation in specific gene expression.

1.3.1. Diet Induced Obesity Models (polygenic models):

It is the most common method to induce obesity in which a diet rich in carbohydrates and fat is consumed by the animals for a long period of time. Many methods have been established for this model. Such as feeding animals high fat diet (HFD) or high fructose corn syrup (HFCS) or a combination of both, exhibited significant increase in the body weight and adiposity compared to animals fed on a normal diet [13-15].

This model of inducing obesity depends on the composition of the consumed diet and on the time in which animals being kept on this special diet to produce obesity.

According to the previous studies, considering the diet type and the period of feeding is crucial as different level of obesity could be obtained as well as the different associated biological changes.

Another way to study obesity-related changes is to use an age related obesity model. This model resembles to human obesity as it characterized by developing a slow, gradual fat accumulation over the individual's life span [16].

Selected breeding of obese animals from dietary obese models is another way to induce obesity. The offspring animals will be having obesity even without being feeding on a high caloric diet. Moreover, the offspring from the mother animals fed on a high caloric diet will have increased obesity risk in their life [16].

1.3.2. Monogenic models:

In these models, a single gene is absent or malfunctioning in the animal. Monogenic mutation in the leptin pathway and Otsuka ling Evans Tokushima fatty rat are examples of what researchers use in genetic inducing obesity models.

1.3.2.1. Monogenic mutations in the leptin pathways:

1.3.2.1.1. Leptin signaling pathways:

Animals usually exhibit a morbid obesity in the presence of defect in the leptin signaling pathway in the hypothalamus of their brains. This defect includes either a lack of leptin production or insensitivity to the leptin through mutations in leptin receptors or resistance to the produced leptin.

1.3.2.1.1.1. Leptin deficiency models:

The ob/ob mouse (obese mouse) is an example of spontaneously mutated genes that leads to the prevention of bioactive leptin secretion. It is characterized by hyperglycemia, hyperphagia, hypothermia, and reduced energy consumption [17].

1.3.2.1.1.2. Leptin receptor deficiency (Leptin insensitivity):

db/db mouse (diabetic mouse) represents an example of a spontaneous mutation in the leptin receptor gene in the presence of elevated leptin levels. It has the same characteristics of ob/ob mouse with more marked hyperglycemia [18]. In addition to that, there is s/s mouse which is genetically engineered animal model of leptin receptor deficiency [19].

The obese Zucker (fa/fa or 'fatty' rat), the Koletsky rat, and Wistar Kyoto fatty rat (WDF rat) are additional examples of leptin receptor-deficient model which are all characterized by insulin resistance and sever morbidity [16].

1.3.2.1.2 Deficit downstream of the brain leptin receptor:

The bioactive leptin works in the brain through targeting two types of neurons in the arcuate nuclei of hypothalamus that has suppressing effects on food intake [20]. These are proopiomelanocortin (POMC) neurons that co-express amphetamine and cocaine, and agouti related protein (AgRP) neurons that that co-express Neuropeptide Y [21].

Due to the fact that POMC is a precursor of α-melanocyte-stimulating hormone (αMSH) in the brain, which increase energy consumption and reduce eating, through activating melanocortin (MC) 3 and 4 receptors, researchers developed Transgenic

mice lacking POMC or POMC knockout mouse models which appeared to exhibit marked obesity [22, 23]. Inversely, AgRP overexpression model has been developed as AgRP increases eating by acting as an antagonist at the MC4 receptor [24]. Moreover, scientists aimed to target the MC receptor themselves and the examples for that; MC4R knockout, MC3R knockout, and MC4/MC3 receptor knockout models [25-27].

1.3.2.2. Otsuka Long Evans Tokushima Fatty (OLETF) Rats:

These rats derived from Long Evans colony that bred at Tokushima Research Institute of Otsuka Pharmaceutical in Japan. They develop mild obesity through the knockout of a cholecystokinin (CCK-1) receptor which has an important role in the satiation. The CCK hormone is produced in the gut and the central nervous system (CNS) and plays an important role in the digestion of food and controlling the appetite and hence satiation. In this model, the animals develop obesity through overeating and increase in the size of the meals and it is considered a valuable model to study the eating dysregulation and obesity [28].

1.4. Obesity management:

Life style changes and medical treatment are the available choices to treat obesity.

1.4.1. Life style modifications:

According to the current guidelines for the management of obesity, life style changes

are considered the first line of treatment with the recommended weight loss of 5%-10% of total body weight in the first 6 months [29]. These changes can be established through reducing the amount of food intake and the dietary calories as well as increasing the physical activity of the individuals [30]. Moreover, behavioral therapy sessions with psychologist are considered beneficial to manage obesity for some subjects [31]. Although these modifications might result in weight reduction over a short period of time, the maintaining of this weight loss is considered somehow difficult on the long term. Subjects having this difficulty are usually advised to participate in the monthly sessions of maintaining weight loss [32].

1.4.2. Medical treatment:

This can be achieved through some medications or the bariatric surgery.

1.4.2.1. Pharmacotherapy:

Some clinicians require pharmacological intervention accompanied with lifestyle changes in some patients with BMI $\geq$30 kg/m^2 and for overweight patients with BMI $\geq$ 27 kg/m^2 who experience obesity complications [33].

There are four appetite suppressants approved by the Food and drug administration (FDA) for short-term therapy usually $\leq$ 12 weeks. These are phentermine, diethylpropion, phendimetrazine, and benzphetamine. The later three drugs prescribed less than the phenteramine. Insomnia and dry mouth are usually the most common side effects of these drugs and they are contraindicated in patients with hypertension

and cardiovascular disease [33].

There are other anti-obesity drugs that regulate the appetite which usually prescribed as long term pharmacotherapy. Such as orlistat, lorcaserin, liraglutide, phentermine/topiramate, and naltrexone/bupropion extended release. These drugs usually have more side effects than the short term medications [33].

1.4.2.2. Bariatric surgery:

Patients who have failed to respond to lifestyle modification and drugs or patients with extreme obesity (BMI $\geq$ 35 kg/m^2 and higher), the bariatric surgery is considered the best treatment option for those individuals [34]. A rise in the advising of these surgeries was reported. There were around 468,609 of bariatric procedure that has been performed worldwide in 2013 with the highest number was around 154,276 in USA and Canada [35]. Even though that they are surgical interventions, they are helpful in alleviating the serious comorbidities usually associated with obesity such as diabetes and insulin resistance, dyslipidemia, and hypertension [36, 37]. Moreover, some studies reported that as a result of theses surgeries, the risk of some types of cancers and mortality rate has been decreased [38].

These surgical interventions can be classified into three major types: 1) sleeve gastrectomy, by removing a large part of the stomach; gastric bypass, 2) through connecting a part of the stomach directly to the intestine bypassing a large part of the stomach and a section of the duodenum, and, 3) laparoscopic adjustable gastric banding, by placing a small band around the upper stomach [33]. The Roux-en-Y

gastric bypass (RYGB) is considered the most frequent procedure performed worldwide (45% of the cases) followed by sleeve gastrectomy (37%) and adjustable gastric banding (10%) but the sleeve gastrectomy procedure is the most common in USA and Canada [35]. Although improvements occur in the obesity associated comorbidities accompanied by the weight loss, adverse events might occur after the surgery. Interrupted disposition of some drugs and nutrient deficiencies due to the removal of some part of the stomach are examples of these adverse effects [39, 40].

1.5. Measures used to quantify body weight and obesity:

WHO recommends to use BMI as an international measure to classify obesity in which people considered to be obese and morbid obese if they have BMI values greater than 30 and 40 kg/m^2 respectively [41]. BMI measure should be considered as a descriptor of body shape rather than a descriptor of body composition because it does not distinguish adipose tissues from muscle masses [42].

Ideal body weight (IBW) measure, which is based on sex and height only, is not indicated to calculate the doses of drugs in obese subjects except for muscle relaxants and remifentanil [43, 44].

Regarding the body surface area (BSA), it is mainly used to calculate the doses for chemotherapeutics drug and it takes into account weight and height [45]. Recent studies reported that the use of BSA-adjusted doses in obese cancer patients neglect the need to reduce or cap the dose in these patients as there is non-linear relation of BSA with the total body weight (TBW) [46, 47].

LBW is most commonly used as a descriptor of body composition which body weight,

height, and gender are required for the calculation [48]. It represents the fat free mass including the weight of muscles, bones, tendons, and organs. However, according to some studies, LBW considered as not always the best predictor for dosing in obesity [49, 50].

In general, in several pharmacokinetic studies in patients with a variety of body weight, TBW was considered to be a good predictor of the drug clearance [49, 50]. Moreover, in a large study of 12 different medications, TBW was a reliable and consistent descriptor for prediction of CL and Vd values in obese patients [51].

1.6. Effect of obesity on drug pharmacokinetics:

Drug pharmacokinetics including; the rate and the extent of absorption, volume of distribution (Vd), and the elimination of the drug from the body (metabolism and clearance (CL)) are the major determinant of the concentration of drugs in the body.

Clearance, which depends on several factors such as drug metabolising enzymes, glomerular filtration, renal tubular reabsorption, renal secretion, and eliminating organ blood flows, is the main parameter to consider in multiple dosing of drugs. Whereas Vd which depends on physicochemical characteristics of drugs, regional blood flow and plasma protein binding, is important to consider in the calculation of loading dose and dose intervals [12].

Due to some pathophysiological changes associated with obesity, drug disposition can be altered and this will cause significant changes in some clinically used drugs pharmacokinetics and pharmacodynamics [12]. The obesity relation with the determinants of drug PK will be discussed in detail.

1.6.1. Effect on Absorption:

Absorption process can be defined as transferring the drug into the central compartment from the site of its administration route [52]. Obesity could affect the oral drug absorption through associated increase in cardiac output, surface area, gastric emptying rate, and gut blood perfusion. However, there are very limited data and research studies on the impact of obesity on absorption of drugs. Studies reported that there is no significant difference in the rate and extent of oral absorption for cyclosporine, dexfenfluramine, midazolam, and propranolol between obese and non-obese subjects [53]. While other research study investigated the oral absorption of midazolam in morbidly obese patients and they found that obesity increased the oral bioavailability of the drug and decreased the absorption rate [11].

With respect to the other routes of administration, the absorption of drugs through subcutaneous, transdermal, and intramuscular routes depends on subcutaneous fat, skin blood perfusion, and muscles respectively. Obesity might affect the rate and extent of absorption of subcutaneous administered drugs due to decreased blood flow rate per gram of fat tissues in morbidly obese subjects. One study showed that the absorption rate of subcutaneously administered enoxaparin, low molecular weight heparin, was decreased in obese subjects with a median time 1 hour longer than non-obese subjects to reach the maximum activity level [54]. However, the extent of absorption was complete in both groups. In contrast, another study reported that obesity did not affect subcutaneous absorption rate of 125 I-labeled rapid acting insulin in type 2 diabetic patients [55].

With intraduodenal administration (i.d), obesity exhibited significant increase in the

absorption of Nelfinavir after i.d administration in obese rats compared to control rats due to the increased bile and decreased expression of intestinal P-glycoprotein which is an efflux transporter [56]. Further research work is needed to conclude the effect of obesity on drug absorption.

1.6.2. Effect on distribution:

Distribution is the transfer of the drugs from the central compartment into different tissues in the body. There are several factors determining drug distribution into various tissues of the body including; body composition, tissues blood flow, plasma proteins binding, and physicochemical characteristics of the drugs such as drug lipophilicity, degree of ionization, and molecular weight. With respect to those factors obesity might have influence on drug distribution as increased adipose tissue mass, increased cardiac output, decreased tissue perfusion, and changed plasma proteins constituents occur in obesity state [57].

Regarding the physicochemical properties of the drug, the lipophilic drugs have higher ability to enter the adipose tissues than the hydrophilic drugs. As a consequence, lipophilic drugs may have a higher Vd in obesity compared to the other hydrophilic drugs. For example, lipophilic drugs; diazepam, verapamil, trazodone, and bisoprolol had a higher Vd in obese subjects than in lean subjects and the Vd of hydrophilic drugs; amikacin, tobramycin, and ranitidine were lower in obese subjects than lean subjects when using a weight-normalized estimates of Vd (Vd /Total Body Weight ratio) to provide a correct measure of how much the drug distribute in to the excess weight as in the obesity, fat tissue mass increase more than the lean tissue mass [58].

Hence, the lipophilic drugs distribute more into adipose tissue and hydrophilic drugs distribute more into lean body tissues. But this is not always the case, caffeine (hydrophilic drug) and Lorazepam (lipophilic drug) had no differences in Vd between obese and lean subjects. Moreover, there are other lipophilic drugs showed decreased Vd in obese populations such as atracrium, digoxin and cyclosporine [58].

Regarding plasma protein binding, studies showed that the albumin, the main protein that acidic drugs bind to, is unaltered in obesity state and there was no change of plasma free level of phenytoin. Whereas α1-acid glycoprotein, the main protein that basic drugs bind to, was increased in the plasma of obese population. Thus, increasing the binding of basic drugs such as propranolol and reducing their free plasma concentration [59]. In addition, in obesity cholesterol and triglyceride levels are increased and hence they might interfere with drugs binding into the lipoproteins and increase their free plasma concentration. Moreover, obesity might cause increased expression of plasma lipoproteins. A study showed significant decreased Vd for nelfinavir and atazanavir in obese rats compared to control rats due to low level of unbound fraction of the drugs caused by elevated plasma triglyceride-rich lipoprotein level [59, 60].

In conclusion, the changes of Vd in obesity state is not only influenced by lipophilicity of the drugs, it is also influenced by plasma protein binding, blood flow, and body composition, and there is a need for more research studies to evaluate the extent of Vd change in obesity to ensure the appropriate drug dosage.

1.6.3. Effect on metabolism:

Metabolism process can be defined as a biochemical transformation of compounds into other forms. This process requires enzymes to convert the non-polar lipophilic compounds to highly polar water soluble metabolites which can be readily excreted into urine or feces. Liver is the main site for the metabolism to occur but it can take place in other tissues such as the kidney and the intestine. Metabolism is covered by two phases; phase I and phase II and each phase involves different enzymes [52].

1.6.3.1. Phase I metabolism:

In this process a polar functional group is introduced into the molecule or modified by oxidation, reduction, or hydrolysis. Phase I reactions may increase or decrease the pharmacological activity of the drugs. Cytochrome P450 (CYPs) enzymes are the main enzymes responsible for phase I reactions [52].

CYPs enzymes are a superfamily of heme-containing enzymes which they are membrane-bound proteins in the smooth endoplasmic reticulum of liver and other tissues including brain, heart, lung, and kidney. They are classified according to their amino acid sequence into families and subfamilies. CYP1, CYP2, and CYP3 families are responsible for the majority of drug metabolism [61, 62].

According to several studies, obesity is reported to cause changes in the expression and activity of some of those enzymes. Some of these reported changes related to each specific enzyme will be reviewed.

1.6.3.1.1. CYP3A:

Cytochrome P450 3A subfamily are recognized to be responsible for about 50% of drug metabolism in the liver and other tissues as they are the most abundant enzymes of CYPs family [63]. Research studies have reported that CYP3A4 had a reduced metabolic activity and all of the CYP3A4 substrates (alfentanyl, midazolam, triazolam, alprazolam, taranabant, carbamazepine, and ciclorporine) showed a significant decreased metabolism in obese subjects and the body weight normalized clearance values is also decreased among obese subjects [64]. In addition, there is a study showed that midazolam systemic clearance was significantly higher after 1 year of performing bariatric surgery in morbidly obese patients [65]. Furthermore, CYP3A4 expression was reduced in the obese animals according to a study on guinea pig models of diet-induced metabolic syndrome [66]. In addition to CYP3A4, Ghose *et al.* showed that diet induced obesity resulted in decreased expression of mice drug metabolizing enzymes CYP3A11, CYP2B10, and CYP2A4 due to reduced gene expression of nuclear receptors [67].

On the other hand, CYP3A2 reported to have increased expression in a study on obese rats induced by high fat diet [68].

1.6.3.1.2. CYP2E:

This enzyme is considered to be responsible for about 5% of liver metabolism [52]. It appeared to be induced in the obesity as several studies showed increased metabolism of the substrates; chlorzoxazone, enflurane, sevoflurane, and halothane in obese subjects versus non-obese subjects obese subjects. Fatty liver infiltration suggested to

be the underlying cause for CYP2E1 induction [64, 69]. In addition, Khemawoot *et al.* while studying the kinetic disposition of chlorzoxazone in obesity, they found that the microsomal activity of CYP2E1 in liver and fat were induced in both Zucker rat fed on high fat diet and genetically obese rats compared to that of control [70].

1.6.3.1.3. CYP1A, CYP2C, CYP2D:

Regarding CYP1A2, patterns of higher drug clearance values are reported in obese subjects which indicate a slight increase in the activity of this enzyme that accounts for 5% of phase I metabolism [64].

CYP2C9 biotransformation is involved in approximately 10% of phase I metabolism. Four substrates of these enzymes have been studied to see the effect of obesity on their pharmacokinetics (Ibuprofen, phenytoin, glimepiride and glipizide). These studies showed that obesity had significantly increased ibuprofen clearance due to increased CYP2C9 activity but not significantly increased metabolism of phenytoin, glimepiride and glipizide. However, bodyweight normalized clearance values showed a slight decrease in CYP2C9 mediated metabolism [64].

CYP2C19 is also approximates 5% of phase I metabolism. Although research studies showed that there was no difference in the metabolism of clorazepate, a substrate of CYP2C19, between obese and non-obese subjects, diazepam metabolism reported to be increased in obesity due to the increased enzyme activity. However, bodyweight normalized clearance values also showed a slight decrease in CYP2C19 mediated metabolism for obese individuals [64].

CYP2D6 that account for 10-15% of phase I metabolism is reported to be increased in obesity state. Dexfeuramine and nebivolol that are substrates for CYP2D6, had higher clearance trend and significant clearance values respectively in obese individuals compared to non-obese individuals [71, 72].

Regarding enzymes other than CYPs that are involved in phase I drug metabolism, Zuccaro *et al.* and Chiney *et al.* investigated the pharmacokinetic of mercaptopurine and caffeine respectively in obese children. These drugs are metabolized by xanthine oxidase enzyme and both drugs had increased metabolism due to increased enzyme activity in obese children [73, 74].

To sum up, phase I drug metabolizing enzymes showed different activities in obesity and the effect of obesity appeared to be enzyme specific.

1.6.3.2. Phase II metabolism:

It is the subsequent reaction in which a functional group is introduced into the parent compound or phase I metabolite in a process called conjugation. Phase II enzymes include various families of conjugating enzymes, such as the UDP-glucuronosyltransferases (UGTs), glutathione-S-transferases, sulfotransferases, *N*-acetyltransferases, methyltransferases, and amino acid conjugation. These enzymes introduce the endogenous substrates such as a glucuronic acid, an amino acid, a sulfate group, or an acetate group to yield a highly polar compound which rapidly excreted from the body [52].

There are controversial results on the effect of obesity on the glucuronidation and sulfation pathways of phase II drug metabolism.

Uridine diphosphate glucuronosyltransferase (UGT 1 and UGT 2) super family enzymes account for 50% of phase II metabolism. Studies showed that obesity exhibited significantly higher UGT enzyme activity on the metabolism of the substrates (paracetamol, garenoxacin, oxazepam, and lorazepam) in obese versus non obese population [64].

In contrast to those studies, there are other data showed that obesity might reduce UGT enzymes expression. Kim *et al.* showed that the expression levels of UGT1A1, 1A6, 2B1 mRNA were significantly decreased in the liver of obese Zucker rats compared to lean Sprague-Dawley rats [75]. Moreover, one recent study showed reduction in expression of hepatic UGT1A1 and enhanced fecal β/glucuronidase activity in diet induced obesity mice administered the chemotherapeutic drug irinotecan and as a result reduced metabolism of SN-38, the metabolite of irinotecan, which this may result in liver toxicity [76].

N-acetyltransferase which accounts for approximately 5% of phase II metabolism has also been investigated in the obesity. Procainamide had a slight increased clearance in the plasma of obese patients by N-acetyltransferase but it was not significant [77]. In addition, Caffeine had a significant increased clearance in obese children compared to non-obese children [74].

Glutathione S-transferase Al-1 isoform which is one of the phase II drug metabolizing enzymes. It is responsible for the metabolism of busulfan. In obesity there was an increase in its activity and a significant increase in its clearance when compared to non-obese subjects. However, when normalized to body weight, its clearance values were lower in obese subjects versus non obese subjects [78].

Sulfotransferase1a1 (SULT1A1) also had a significant reduced expression in mice fed on high fat diet compared to their lean controls due to an induced inflammation cell signaling [67].

In summary, several studies showed that the expression of metabolizing enzymes can be altered by obesity. Enzymes expression changes could affect the metabolism of various endogenous and exogenous compounds. With respect to used drugs, if the metabolizing enzyme levels increase, this will increase its clearance and reduce the therapeutic effect of drug. In reverse, if the enzyme levels decrease, the plasma concentration of drug will increase and consequently this might lead to toxic effects.

1.6.4. Effect on renal excretion:

The kidney is the major organ responsible for the excretion of drugs. Renal excretion involves one of the three processes: glomerular filtration, passive tubular reabsorption and active tubular secretion [52]. Obesity might have an influence on renal clearance through changing the rate of glomerular filtration or tubular secretion.

1.6.4.1. Glomerular filtration:

There are few studies investigated the impact of obesity on drugs that eliminated by glomerular filtration. Kosmisky *et al.* reported that vancomycin clearance was increased with total body weight in morbidly obese individuals and the majority of patients had sub therapeutic concentrations which this may lead to increase the risk of resistance and treatment failure [79]. Daptomycin clearance also significantly increased with people having higher mean total body weight (126kg) but not with people with a mean total body weight of 114kg [80, 81]. However, normalized to body weight clearance values showed equal or lower clearance values when compared to normal weight individuals. While those studies showed enhanced glomerular filtration rate in obesity state, there was no effect of obesity on clearance of cimetidine in obese patient and the glomerular filtration rate was not altered [82].

1.6.4.2. Tubular secretion:

About the half of administered procainamide dose is excreted unchanged by glomerular filtration and tubular secretion. Procainamide had higher renal clearance in obese patients as a result of elevated tubular secretion and there were no significant differences between obese and non-obese patients in 24-h creatinine clearance indicating no differences in glomerular filtration [77]. Ciprofloxacin and cisplatin (eliminated mainly by tubular secretion) also had higher clearance values in obese patients [47, 83]. Digoxin showed a trend toward a higher clearance in obese subjects probably because of elevated tubular secretion. However, the normalized to body

weight clearance values for tubular secretion in obese patients reported to be equal or slightly lower than non-obese patients [84].

1.7. Effect on drug transporters:

Transporters are proteins that play important roles in drug absorption, distribution, metabolism and excretion. They are either: influx proteins that facilitate the uptake of drug from extracellular into intracellular space, and efflux proteins that take the drug outside the cell [85]. Obesity can cause some changes in various drug transporters expression that are noticed both in humans and animals.

1.7.1. ABC family:

The adenosine triphosphate binding cassette (ABC) transporter superfamilies bind ATP and utilize the energy to translocate a wide variety of substances across intracellular and extracellular membranes. This family can be classified according to amino acid homology and domains organization into seven distinct subfamilies from A to G to include 48 known proteins in total [86].

1.7.1.1. P-glycoprotein:

P-glycoprotein (P-gp), the permeability glycoprotein or plasma glycoprotein which belongs to ABC super family is an active, efflux, membrane bound transport protein pump. It is encoded by multidrug resistant gene 1 (MDR1) in humans. It is also abbreviated as ABCB1and PGY1. The gene shows an exclusive over expression in

cancer cells. Multidrug resistance (MDR) phenotype occurs through up regulation of MDR1 gene mRNA transcription and over expression of the P-gp transport system during the drug therapy for cancer and several microbial infections [87]. In addition to anticancer drugs, various therapeutic agents that act on CNS, cardiovascular system and antimicrobials are substrates to this protein. P-gp can be found in various tissues including liver, pancreas, small and large intestines, jejunum, colon, kidney, and blood-brain barrier (BBB) [87].

Regarding the influence of obesity, many studies reported different changes in the expression of P-gp. Ghoneim *et al.* found an induction in MDR1 hepatic expression in obese rats fed on high fat diet compared to control rats [68]. In addition to the induction in the hepatic expression levels, it is found to be also increased in the intestine of obesity-induced hyperglycemic mouse model [88]. Conversely, Sugioka *et.al* reported a significant decrease in both liver and intestinal P-gp expression while studying the effects of obesity induced by high-fat diet on the pharmacokinetics of nelfinavir [56].

1.7.1.2. MRPs:

Multidrug resistance protein (MRP) subfamily is also an efflux transporter that belongs to the ABC transporters family. MRPs mediate the efflux of a broad range of anionic compounds as well as glutathione and glucuronide conjugates. There are nine structurally and functionally related family members from MRP1 to MRP 9 and they differ in their localization, expression levels, and substrate specificity. Similar to P-gp,

MRPs confer resistance to chemotherapeutic drug while transportation though over expression [89].

MRPs transporters have been reported to be influenced by obesity. More *et al.* investigated the expression levels of both hepatic and renal transporters in diet induced obese mouse and they found that the HFD increased the expression of MRP3 and MRP4 mRNA and protein in liver by 3.4 and 1.4 fold respectively compared to control mice fed a low-fat diet (LFD) [90]. Conversely, mRNA and protein of MRP1 decreased by half in livers of obese mice compared with those in lean mice livers. In kidney, the HFD did not change the expression levels of transporters compared with the LFD [90]. Other study showed that obesity exhibited significant reduction of hepatic MRP2 that plays an important role in the biliary excretion of drugs [91].

1.7.2. SLC family

Solute carrier (SLC) family is a superfamily that facilitates the uptake a variety of substrates into cells. This family classified into 52 subfamilies and includes 395 membrane-bound proteins in total [92].

1.7.2.1. OCTs:

Organic cation transporters (OCTs) are facilitated diffusion transporters that are members of solute carrier 22 A family (SLC22A). They are expressed in kidney, liver, placenta cells, and brain to contribute to the uptake of various physiological compounds and organic cation xenobiotics in mammals [93].

Studies reported that obesity can affect the expression of OCT 1 and OCT 2. Jang *et al.* revealed that hepatic uptake of metformin which is an OCT1 substrate was significantly higher in obese mice fed on high fat diet compared to lean mice. Their results were consistent with their finding of higher expression of OCT1 mRNA in obese mice [94]. In addition, other study investigated the gene expression and protein level of OCT1 in adipose tissues and during adipogenesis in obese subjects. Researchers found significantly higher expression level of OCT1 protein in subcutaneous and visceral adipose tissues [95]. In contrast to OCT1, OCT2 has been found to have lower expression levels in obese diabetic rats [96].

1.7.2.2. OATs:

Organic anion transporters (OATs) are also efflux transporters and members of Solute Carrier Family 22 (SLC22). OATs play a vital role in renal excretion of negatively charged organic compounds including endogenous waste products, drugs and drug metabolites. OATs are can be found in kidney, liver, placenta, nasal epithelium, and liquor-brain barrier [97].

Regarding the influence of obesity, the ob/ob mice in Cheng *et al.* research study exhibited a significant reduction in renal OAT2 mRNA expression levels [98].

1.7.2.3. OATPs:

Organic anion transporting polypeptides (OATP) proteins are en efflux transporters for various endogenous and drug substrates and they belong to SLCO transporter

family. OATPs are expressed in various tissues including liver, kidneys, and intestine [99].

Obesity has been associated with different changes in OATPs expression. For example, upon studying the organic anion transporters regulation in obese Zucker rats, OATP2 has been found to have significant reduction in mRNA and protein expression levels in these rats [91]. Moreover, Cheng *et al.* reported that OATP1A1 mRNA and protein expression in livers of ob/ob mice were reduced as well as significant reduction in the mRNA expression levels of OATP1A1 in kidney compared to the wild type mice [98]. In contrast to those findings, a research study showed that obese rats fed on high fat diet exhibited higher hepatic expression protein levels of OATP1A4 compared to their control [68].

In summary, obesity can have different effects on drug transporters. This might affect drugs pharmacokinetics and hence their pharmacodynamics.

1.8. Effect on nuclear hormone receptors:

Nuclear hormone receptors (NHRs) are ligand-dependent transcription factors that function as modulators of tissues gene expression. The NHR superfamily contains 48 genes in humans, 49 in mice. The ligands of NHRs are always lipophilic and variable in size and structure. Examples of these ligands, include glucocorticoids, steroid hormones, fatty acids, phospholipids, heme, bile acids, vitamin D and xenobiotics [100].

1.8.1. CAR:

The constitutive androstane receptor (CAR) belongs to subfamily NR1I of the nuclear receptors superfamily. CAR is mainly expressed in the liver and kidneys and in low levels in brain, intestine, and heart. CAR activation alters lipid metabolism and glucose homeostasis. In addition, CAR regulates detoxification and excretion of toxic endogenous metabolites, such as bilirubin and bile acids. Moreover, CAR can be also activated by xenobiotics to regulate expression of genes encoding proteins involved in their metabolism and elimination including cytochromes P450, UDP-glucuronosyl transferase, sulfotransferases, and proteins of multiple drug resistance [101].

Ghose *et al.* studied the effect of high fat diet on gene expression of drug metabolising enzymes and transporters in mice. They found that the RNA and protein levels of CAR were reduced approximately 60% in the livers of mice fed on high-fat diet compared to the control LFD mice with a consequent reduction in CYP2B10, CYP2A4 RNA levels as these enzymes regulated by CAR only in mouse [67].

1.8.2. PXR:

Pregnane X receptor (PXR) belongs subfamily NR1I2 of the nuclear receptors superfamily. PXR is mainly expressed in the liver, small intestine and kidneys. It also expressed in low levels in colon, prostate, brain, breast, heart, bone marrow, ovary, and placenta. PXR plays an important role in glucose and lipid metabolism, bile acid and bilirubin detoxification, steroid hormone homeostasis, vitamin metabolism and inflammation. In addition, PXR can be also activated by xenobiotics to regulate the expression of genes that encode proteins involved in their metabolism and elimination

including cytochromes P450, UDP-glucuronosyl transferase, glutathione S-transferase, sulfotransferases, and proteins of ABC and SLC families [102].

Several studies reported that obesity can cause changes in the PXR expression. Upon studying the effect of high fat diet on the nuclear receptor hepatic expression and their target genes in rats, Ghoneim *et al.* stated that along with increase in body weight and body fat, the rats exhibited a 2-fold increase in PXR mRNA expression as well as significant expression of hepatic PXR target genes Abcc2, and CYP3A2, MDR1, and MRP2 [68]. However, Ghose *et al.* reported a different exhibition where they found a significant reduction in the expression of PXR mRNA and protein and PXR target genes (CYP3A11 and UGT1A1) in mice fed on high fat diet compared to LFD mice [67].

1.8.3. FXR:

Farnesoid X receptor (FXR, NR1H4) as a transcription factor, regulate genes involved in bile acid and cholesterol synthesis, metabolism, and excretion [103]. FXR regulate bile acid homeostasis by promoting bile acid and phospholipid biliary secretion through the induction of the bile salt export pump (ABCB11 and ABCB4). FXR activation also suppresses the Na-dependent taurochlorate co-transporting protein (SLC10A1 (Na/bile acid co-transporter)) mediated uptake of bile into the liver [104]. Furthermore, FXR plays a role in regulating genes involved in lipid metabolism, insulin regulated pathways, and inflammatory response [103]. FXR is highly expressed in the liver, intestine, kidney and adrenals, but with lower extent in the adipose tissues and heart [105].

According to Ghoneim *et al.*, hepatic mRNA level of FXR was significantly increased in rats fed on HFD. In the same study, they reported a 2- and 3-fold increase in the mRNA levels of ABCB11 and ABCB4, respectively compared to their control. In addition, SLC10A1 expression level was found to be significantly increased in the HFD group. However, the expression level of CYP7A1, which is involved in cholesterol synthesis, was not significantly different between the groups [68].

In contrast to the previous study, mRNA of FXR expression level was significantly reduced in obese rats model with insulin resistance compared to the rats fed on normal diet [106].

1.9. Obesity and inflammation:

There are two available types of adipose tissue: brown and white adipose tissue. While brown adipose tissue provides energy through non oxidative phosphorylation reactions in the form of heat to adapt in cold conditions, white adipose tissue provides energy for metabolic functions through oxidative phosphorylation reaction as the white adipose tissues provides most of the total body fat and they are the source of fatty acids that are used as energy substrates for generation of adenosine triphosphate (ATP). White adipose tissues contain genes responsible for secretion of inflammatory mediators [107].

In obesity state, the white adipose tissue amount increases followed by an increase in inflammation levels that can be described as low-grade form of inflammation. In obese patients, there is an increased circulating level of several inflammatory markers such as CRP (C-reactive protein), tumor necrosis factor α (TNFα), and interleukin-6

(IL-6), IL-18, macrophage migration inhibitory factor (MIF), haptoglobin, serum amyloid A (SAA) and plasminogen activator inhibitor 1 (PAI-1) [108].

It has increasingly been considered that the consequences of obesity inflammatory state especially the production of inflammatory adipokines, can lead to the development of the diseases associated with a high BMI such as Type II diabetes and atherosclerosis. In addition, there is a growing recognition of the importance of inflammation as a factor in the causes of a wide range of diseases, including diseases associated with aging such as the dementia [108].

1.9.1. Inflammation and altered expression of metabolizing enzymes and drug transporters:

The increased plasma cytokines levels in obesity can be contributed to the increase in adiposity as well as macrophage infiltration. Several studies suggested that the increase in plasma inflammatory cytokines can cause change in expression of some metabolizing enzymes and transport proteins due to cytokines effect on the nuclear receptors (such as PXR and CAR) that regulate their expression [109].

Morgan et al. did extensive review on the regulation of CYP450 under infection and inflammation conditions. In studies of the induced inflammation in rats and mice by injecting lipopolysaccharide (LPS), they demonstrated an increase in the level of inflammatory cytokines such as TNFα with the accompanied decrease in the expression of hepatic CYPP450 mRNAs such as *CYP1A2, 2A1, 2C6, 2C7, 2C11, 2C23, 2E1* and *3A2* in rats as well as the decreased expression of the mRNAs of *Cyp1a2, 2a5, 2c29, 2e1*, and *3a11* in the mice [110]. They attributed this effect to the

macrophages filtration where it releases IL1-β and TNFα which in turn lead to secretion of IL-6 and chemokin from the other cells types, eventually nitric oxide and reactive oxygen species will be produced and irreversibly modify the CYP450 proteins [110]. Moreover, Aitken *et al.* studied the effect of IL-6, TNFα, interferon γ (IFN), transforming growth factor-β (TGF) and interleukin-1β (IL-1) treatment on expression of CYP450 mRNA in human hepatocytes. They found that CYP3A4 and CYP2C8 were down-regulated by all cytokine treatments and the other CYP2Cs and CYP2B6 were down regulated according to the type of cytokine treatment [111].

The effect of inflammation on UDP-glucuronyl transferase isoforms (UGT) has been studied as well. In one study, upon an induction of inflammation by using LPS in mice, researchers reported that hepatic mRNA expression of UGT1A1, 1A9, and 2B5 was down-regulated, whereas hepatic UGT1A2 and 1A6 mRNAs were unchanged as well as the renal UGT isoforms except for renal UGT2B5 where it was induced [112]. The researchers demonstrated that this effect could be cytokine-dependent.

Regarding the influence on transporter proteins, one study reported that the exposure of human hepatocytes to IL-1β was shown to significantly down-regulate the mRNA levels of influx transporters OATP-B, OATP-C, and OATP8 and the efflux pumps MRP2, MRP3, MRP4, and breast cancer resistance protein as well as reduced bile salt export pump (BSEP) and OATP-C protein expression [113]. The same researchers in the previous study also investigated the effect of proinflammatory cytokines TNF-α or IL-6 exposure on the expression of some transporters in human hepatocytes. They

found that both cytokines down-regulated mRNA levels of influx transporters OATP1B1, OATP1B3, OATP2B1, OCT1 and 2 [114]. According to their effect on the efflux drug transporters, while IL-6 was also found to decrease mRNA and protein expression of MRP2 and BCRP, TNF-α markedly reduced bile salt export pump mRNA levels and increased BCRP protein expression [114].

1.10. Effect of obesity on pharmacodynamics (PD) of drugs:

PD can be defined as the relationship between drug concentrations and pharmacological effects [115].

Limited data is available on the effect of obesity on drugs PD. Physiological and genetic changes related to obesity might affect receptor expression and receptor affinity to drugs and as a result affecting their PD [116].

Obese patients have shown increased sensitivity for to a benzodiazepine derivative drug, triazolam, which was measured by a sedation score, compared to non-obese individuals [117]. Schmid *et al*. reported that there was a trend toward significantly increased dose requirements for analog insulin in patients with BMI >30 kg/m^2 to reach target glucose levels [118]. Furthermore, there is a study investigated the clopidogrel (antiplatelet drug) efficacy in obese and non-obese patients undergoing angioplasty and stenting for cardiovascular disease. The researchers found significant poor response to clopidogrel as well as increased platelet activation in obese compared to non-obese patients [119].

Although some medications have already established dosage adjustment for obese patients, the preliminary findings of the effect of obesity on PK/PD demands further

studies and evaluation of obesity effect on drug therapy especially for medications with narrow therapeutic effect as to enhance the knowledge about drugs efficacy and safety in obese patients [58, 120].

1.11. Amiodarone:

The interest in the antiarrhythmic drug amiodarone (AM) is increasing because of its reported efficacy in treating several types of arrhythmia and its variety of adverse side effects. AM is a lipophilic benzofuran derivative and it was initially developed to treat angina pectoris in patients having coronary artery disease. Then AM revealed it to be a very effective in treatment of ventricular and supraventricular tachyarrhythmias [121]. AM classified as class III anti arrhythmic agent according to the Vaughan Williams classification system and it is available in oral formulations and in case if more prompt effect is required it can administered intravenously [122].

1.11.1. Pharmacological actions of AM:

Amiodarone is considered as potassium channel blocker (class III antiarrhythmic), which prolongs phase 3 and as a result prolonging the effective refractory period. AM also can act as sodium and calcium channel blocker as well as decreasing beta-adrenoreceptors producing beta blockade. AM principal effects are the delay in repolarization by prolonging the action potential duration as well as effective refractory period [123]. Aside from its effect on the conduction system, AM can act as vasodilator as a result of its calcium antagonistic properties and thereby improves coronary perfusion and decreases peripheral resistance [122].

1.11.2. Pharmacokinetics of AM:

The time to peak amiodarone concentrations after oral dosage is relatively long (3-7 hours) and the estimates of bioavailability are about 20-80% and it undergoes extensive enterohepatic circulation before distribution to central and tissue compartments [124]. AM is highly protein bound mainly to albumin (62.1%) and small amounts to beta lipoprotein (33.5%) it has a Vd of about 66L/kg in human [125]. Tissues high in fat content, such as adipose tissue, liver, and heart are the sites of high amiodarone concentrations [126].

AM has an extended terminal phase half-life ($t_{1/2}$) for days and very slow elimination and thereby it given once daily [127]. Furthermore, AM has a low hepatic extraction ratio in humans and a moderate hepatic extraction ratio in rat [128, 129].

AM therapeutic plasma concentration is between 0.5–2 μg/mL. Before starting the maintenance dose of 200-400 mg/day high loading dose of 1200–1600 mg/day is needed for several weeks because the large volume of distribution of the drug would delay the time to achieve minimal effective drug concentrations [130].

AM elimination occurs mainly via hepatic oxidative metabolic pathways with negligible renal elimination in both human and rats [129, 131]. The metabolism in liver results in the major active metabolite *N*-desethylamiodarone (DEA) by CYP3A4, 1A1, 1A2, 2D6 and 2C8 in human and CYP3A1 and 1A1 in rats [132]. DEA has been reported to be equipotent as a sodium channel blocker and less potent as a calcium channel blocker compared to AM. In addition, DEA reported to be less protein-bound than the parent drug and it's more available for distribution to the heart. Other minor metabolites of AM have been reported, including deiodinated forms [133].

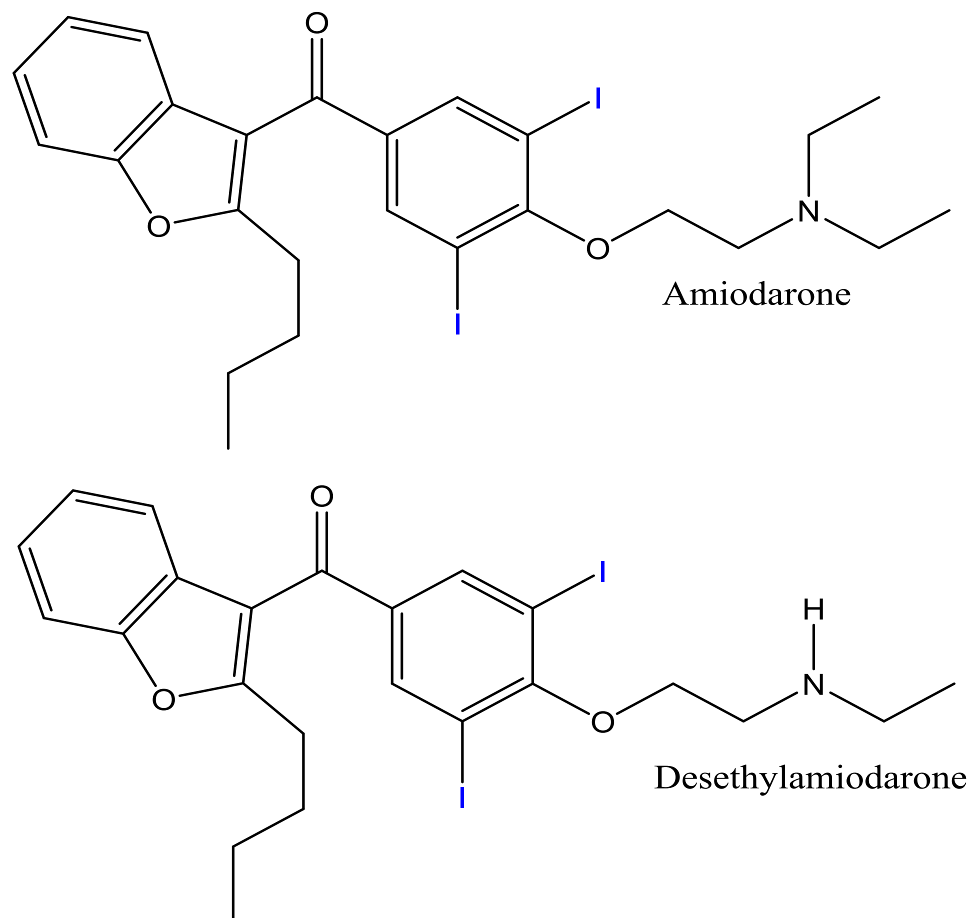

Figure 1: **Chemical structure of amiodarone and desethylamiodarone.**

AM is associated with many adverse effects and pulmonary fibrosis being the most serious side effect [130]. Studies reported that an existing correlation between pulmonary toxicity and higher DEA:AM serum concentration ratios in patients receiving AM[134]. Other adverse effects were reported including; hepatic

dysfunction, corneal epithelial deposits, peripheral neuropathy, proximal muscle weakness, and symptoms of thyroiditis and thyrotoxicosis [130].

AM being metabolized by the CYPs can cause a number of drug interactions. Amiodarone inhibits p-glycoprotein and CYP1A2, CYP2C9, CYP3A4 and CYP2D6 enzymes resulting in increased concentration of other drugs metabolized via these pathways. For example, warfarin, digoxin, simvastatin, β-blockers, calcium channel blocker, and Class I antiarrhythmic drugs. And due to long elimination half-life that AM have, these effects may persist for a long period of time [125].

1.12. Bisphenol A (BPA):

BPA is a synthetic lipophilic xenoestrogens that is capable of disrupting endocrine functions by mimicking the endogenous hormones**.** BPA is heavily used in the industry for synthesis of polycarbonates, epoxy resins and thermal paper thus it commonly appears in various products including medical equipment, dental products, electronic devices, CD/DVD discs, water-pipes, paper or toys. Bisphenol A is also used in food contact materials including packaging, bottles and coatings leading to exposure of consumers to BPA through food and drinking water [135].

1.12.1. Pharmacological actions of BPA:

BPA binds to several kinds of receptors. BPA binds to estrogen receptors (ERs) producing weak estrogenic activity as it has 1000 to 2000 fold less affinity to the ERs than estradiol. In addition, BPA is able to bind to androgen receptors, aryl hydrocarbon receptor, thyroid hormone receptor, and peroxisome proliferator-

activated receptor that are associated with hormones of the endocrine system of the body [136].

BPA due to endocrine-disruption activity is reported to disrupt the fertility and reproduction of both male and female. In addition, it has been indicated that BPA disrupts the function of various hormones such as sex hormones, leptin, insulin and thyroxin and causes hepatotoxic, immunologic, mutagenic and carcinogenic effects. Recent data also suggested that human exposure to BPA elevate the risk of obesity, type 2 diabetes and cardiovascular disease. Moreover, recent studies proven that there is association of BPA with oxidative stress, inflammation, and altered epigenetic markers and gene expression [137].

1.12.2. BPA pharmacokinetics:

Following oral administration, the majority of BPA is quickly bound to glucuronic acid by UDP-glucuronosyltransferase to produce BPA glucuronide (BPA-G) in the liver and gut and the levels of unconjugated BPA in blood are less than 1% of the total administered dose and more than 90% of BPA excreted in urine as conjugated metabolites. To a lesser extent, unconjugated parent BPA is converted to other metabolites, primarily BPA sulfate [138]. BPA binds to plasma proteins in humans with the bound form representing around 90–95% and the free form 5–10% of the total [139].

Shin *et al.* developed a physiologically based pharmacokinetic (PBPK) model to predict the tissue distribution and blood pharmacokinetics of BPA in rats and humans where BPA was administered by rats through multiple intravenous injections to steady

state and in human as single intravenous injection and multiple oral administrations to steady state [140]. The average steady-state concentration of BPA was found higher in tissues than corresponding blood levels and the small intestine had the highest concentration. The values of CL, and $t_{1/2}$ predicted for a 70-kg human were 116.6 L/h, and 76.8 min, respectively which were similar to the values predicted in the literature by simple allomertic scaling in rats.

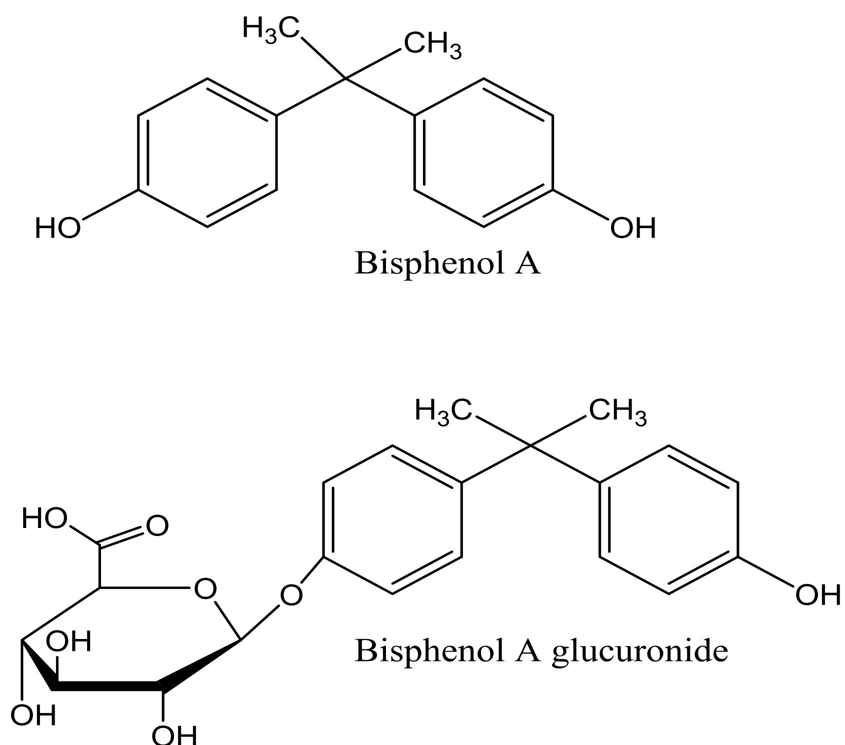

Figure 2: Structure of BPA and its metabolite BPA glucuronide.

1.13. Rationale, Hypotheses, Objectives:

1.13.1. Rationale:

Obesity is considered to be a leading cause of death in developed countries and the incidence for the associated serious medical conditions is rising. Obesity can be associated with several comorbidities including cardiovascular diseases, insulin resistance, type 2 diabetes, atherosclerosis and hyperlipidemia. As a result, obese subjects having such comorbidities are required to take medications to treat them. Although several studies have investigated the drug disposition in obese patients, the knowledge about the PK and drug disposition and the subsequent impacts on pharmacodynamics of medications in obese individuals remains limited.

According to the literature, the VD of some drugs especially lipophilic drugs could be changed in obesity due to increased adiposity as well as the change in some drugs clearance. These changes would have an impact on the pharmacological responses of drugs as it may result in reduction in the therapeutic efficacy or appearance of toxicological effects.

As known, the majority of medication undergoes biotransformation by metabolizing enzymes or transportation by transporter proteins. Although several research studies have indicated that the expression of these metabolizing enzymes and transporters is altered in obesity, very few studies has been done to see if obesity would change their functional activity.

Another factor to explore is that the associated inflammation process and the resultant secretion of pro-inflammatory cytokines have been indicated to have an impact on the expression of metabolizing enzymes and drug transporters.

Furthermore, the presence of hyperlipidemia (HL) and the increase in the plasma lipoprotein such as triglycerides (TG), low-density lipoproteins (LDL), and free fatty acids with obesity can have an effect on the distribution of drugs and subsequent metabolism and excretion. HL has been shown to change the PK of drugs that are bound to plasma lipoproteins. It can limit drug uptake into the cells and decreases the unbound fraction of drugs through increasing their binding to the plasma lipoproteins, thereby decreasing their metabolism. In addition, and it is reported to decrease the metabolism and hepatic CL of low and moderate hepatic extraction ratios drugs [128, 141, 142].

1.13.2. Hypotheses:

1. Dietary induced obesity can cause a decrease in the functional activity of CYP450 enzymes in liver and intestine.

2. Dietary induced obesity can cause an increase in the functional activity of glucuronidation enzymes in liver and intestine.

3. Genetically obese rats might have changes in the functional activities of CYP450 and glucuronidation enzymes in liver.

1.13.3. Objectives:

1. To study the impact of dietary induced obesity on the functional activities of CYP450 in the liver and intestine.

2. To study the impact of dietary induced obesity on the functional activities of UGTs in the liver and intestine.

3. To study the impact of genetic obesity on the functional activities of CYP450 in the liver.

4. To study the impact of genetic obesity on the functional activities of UGTs in the liver.

Chapter2: Materials and methods

2.1. Materials:

Amiodarone HCl (AM), ethopropazine HCl, nicotinamide adenine dinucleotide phosphate tetrasodium (NADPH), sodium carbonate, sodium thiocyanate, sodium hydroxide and bovine serum albumin were purchased from Sigma-Aldrich (St. Louis, MO). Desethylamiodarone (DEA) was obtained as a gift from Wyeth Research (Monmouth Junction, NJ). Sulfuric acid was purchased from Caledon Laboratories Ltd (Georgetown, Ontario, Canada).

Methanol, hexane, acetonitrile, triethylamine, and methyl tert-butyl ether/oxyde (all HPLC grade) were purchased from EM Scientific (Gibbstown, NJ). Potassium phosphate monobasic, magnesium chloride hexahydrate, and sucrose (all analytical grade) were obtained from BDH (Toronto, ON, Canada).

Bisphenol A was purchased from Sigma-Aldrich (St. Louis, MO) and naproxen was purchased from syntax laboratories (California, U.S.A)

2.2. Stock solutions:

Stock solutions of 50mg/mL of AM, 0.1 mg/mL of DEA and ethopropazine as an internal standard (IS) were prepared. In addition, 0.1 mg/mL stock solution of BPA was also prepared (all in methanol). While AM and DEA standard curves were prepared in several concentrations ranging from 0.05 to 100 µg/mL, the BPA standard curve ranged from 0.025 to 10 µg/mL. All stock solutions were stored at -20 ºC.

2.3. Methods:

2.3.1. Animal model of obesity:

The source of the microsomal protein were from the same rats previously published [143]. In brief, Sprague-Dawley male rats aged 4 weeks were fed three different dietary components. Each group consisted of 10 rats fed the diets for 14 weeks. The diets were either a high fat diet as rat pellets (45% kcal of fat, Harlan Laboratory, Inc.) with normal water or a 13% w/v high fructose corn syrup (HFCS) (prepared in our lab) with standard rat chow or a combination of both (HFCS-HFD) with the control group being fed on a standard rodent diet (13.4 % kcal of fat) and normal drinking water.

Regarding JCR, lean and genetically obese animals were fed the same diet from Proctor lab consisted of standard rodent chow mixed with 30% (weight for weight) lipid. Lipid comprised of flaxseed oil, tallow, sunflower oil and olive oil yielding saturated to poly-unsaturated fatty acid ratio of 1:1. Cholesterol 1% (w/w). Carbohydrate 49% (w/w), protein 28% (w/w), moisture 10%, minerals (4%) and fibre (6%).

The livers and intestines of these rats were collected after they were euthanized. All tissue specimens were kept at -80 °C until needed.

2.3.2. Preparation of Microsomes:

Pooled livers and intestine tissues of all groups (n=3) rats were homogenized in cold sucrose solution (0.25 M in distilled water) by using a homogenizer (0.5 g of each tissue in 2.5 mL of sucrose). The homogenate was centrifuged at 1000 g for 10 min to

remove nuclei and large cellular debris. The supernatants were transferred to new tubes and centrifuged again at 15,000 g for 10 minutes to remove organelle fraction. Centrifugation continued again by transferring supernatants to new tubes at 100,000 g for 1 hour to precipitate the microsomes. Then the pellets were resuspended in sucrose 0.25 M solution and stored at -80° C [144].

2.3.3. Lowry assay for protein concentration in microsomes:

The Lowry assay method for protein concentration is based on comparing the unknown concentration of protein preparation with serial standard solutions of bovine serum albumin (BSA). The following solutions required to be prepared In order to assay the concentration of protein in microsomal preparations: Reagent A: 1mL of sodium and potassium tartarate 2% in distilled water, 1mL of $CuSO_4$ 1% in distilled water, and 20 mL of Na_2CO_3 anhydrous 10% in 0.5 M NaOH.

Reagent B: 1:10 diluted solution of folin-phenol reagent in distilled water. Working standard solutions of BSA were prepared at the concentrations of 500, 400, 300, 200, 100, and 0 μg/mL of BSA in distilled water from the stock solution of 500 μg/mL (50 mg/100 mL H_2O). To a number of clean test tubes containing 2 μL of microsomal preparation and 248 μL of distilled water (unknown concentration of protein) or 250 μL of each standard solution, 250 μL of reagent A were added and the tubes were incubated at room temperature for 10 min. In the next step while continuously vortex mixing, 750 μL of reagent B was added to each of the test tubes and samples incubated at 50°C for 10 minutes. At the last step 200 μL of each mixture were

transferred to a well in the ELISA plate and analyzed using a plate reader at 550 nm [145].

2.3.4. Microsomal incubation of control and obese rats with AM:

The formation kinetics of DEA were characterized when AM was exposed to pooled liver and intestine microsomes of control and high caloric diets fed rats as well as the microsomes of lean and obese JCR rats. Each 0.5 mL incubate contained 1 mg/mL of protein for the liver and 1 mg/mL of protein in case of intestine and JCR. Each sample also contained 5 mM magnesium chloride hexahydrate dissolved in 0.5 mM potassium phosphate buffer (pH=7.4). For the incubations, AM HCl was added to provide a nominal concentration of 5, 20, 40, 80, and 155 μM. For this, AM HCl was dissolved in methanol such that a total methanol concentration of 0.8% was present in each incubation mixture. The reaction was started with the addition of 1 mM NADPH after a 5 min pre-equilibration period. All incubations were performed at 37ºC in a shaking water bath (50 rpm) for 10 min. The oxidative reaction was ended by addition of 1.5 mL ice-cold acetonitrile. Samples were kept at -20º C until assayed for AM and DEA by HPLC [146].

2.3.5. Microsomal incubation of control and obese rats with BPA:

The consumption kinetics of BPA were characterized when BPA was exposed to pooled liver and intestine microsomes of control and high caloric diet-fed rats as well as the microsomes of lean and obese JCR rats. Each 0.5 mL incubate contained 1 mg/mL of protein for all microsomes and 5 mM magnesium chloride hexahydrate

dissolved in 0.5 mM potassium phosphate buffer (pH=7.4). To incubations, BPA was added to provide a nominal concentration of 0.5, 2, 5, 10, 25, and 50 µM. For this, BPA was dissolved in methanol such that a total methanol concentration of 0.8% was present in each incubation mixture. The reaction was started with the addition of 10 mM UDPGA after a 5 min pre-equilibration period. All incubations were performed at 37ºC in a shaking water bath (50 rpm) for 10 min [147]. The glucuronidation reaction was ended by addition of 100 µL of 1M HCL. Samples were kept at -20º C until assayed for remaining BPA by HPLC.

2.3.6. Confirming the BPA glucuronidation process by adding β-glucuronidase enzyme:

BPA was added in a concentration of 10 µM to triplicate incubates. Each contained 1 mg/mL of protein of pooled hepatic microsomes of control rats and 5 mM magnesium chloride hexahydrate dissolved in 0.5 mM potassium phosphate buffer (pH=7.4) in a total volume of 0.5 mL. The reaction was started with the addition of 10 mM UDPGA after a 5 min pre-equilibration period. The incubation was performed at 37ºC in a shaking water bath (50 rpm) for 10 min. After that, 200 Unit of β-glucuronidase enzyme was added to the incubations and left in the shaking water bath for 6 hours for the conjugated drug to be hydrolyzed back to BPA [148]. Then 100 µL of 1M HCL was added and samples were kept at -20º C until assayed for BPA concentration by HPLC.

2.3.7. AM and DEA assay:

A published HPLC method was used for analysis of AM and DEA [149, 150]. The working standard solutions were prepared daily from the stock solution by serial dilution with methanol to give final concentrations of 500, 1000, 5000, 20,000, 50,000, and 100,000 ng/mL of AM and 50, 100, 500, 1000, 2000, and 5000 ng/mL of DEA.

2.3.8. Extraction Procedure:

Validated and published extraction procedure was followed [149, 150]. In brief, 30 µL of internal standard (ethopropazine HCl) was added to all samples. Then the samples were vortexed-mixed for 30 s and centrifuged for 5 min at 2500 g to ease protein removal. Thereafter, 6 mL of hexane was added to the supernatant layers in new test tubes and the mixture was vortex mixed for 45 s, then centrifuged for 5 min. The final organic layer was transferred in to new tubes, evaporated to dryness, reconstituted in 150 µL mobile phase and 50 µL was injected into the HPLC apparatus.

2.3.9. HPLC conditions for AM:

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.). The C8 analytical column was used (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 55:12:33 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 internal standard. After 4 min post-injection, it was switched to 242 nm which represents the UV maximum of AM and DEA.

2.3.10. Extraction Procedure for BPA:

In brief, 30 μL of internal standard (naproxen) was added to standard solutions, control, and treated samples. Then the samples were vortex mixed for 30 s and centrifuged for 5 min at 2500 g to ease protein removal. Thereafter, 6 mL of methyl tert-butyl ether was added to the supernatant layers in new test tubes and the mixture was vortex mixed for 45 s, then centrifuged for 5 min. The final organic layer was transferred in to new tubes, evaporated to dryness, reconstituted in 150 μL mobile phase and 100 μL was injected into the HPLC apparatus.

2.3.11. HPLC conditions for BPA:

The HPLC system consisted of a Waters 717 auto sampler, Waters 600 multi-solvent delivery system, and Waters 486 tunable absorbance detector. Data collection and integration were accomplished using Ezchrom software computer based integrator.). A C18 guard column and analytical column was used (250 mm×4.6 mm with 5μm particle size) for separation. The mobile phase consisted of acetonitrile: phosphate buffer [25 mM KH_2PO_4: 3 mM H_2SO_4: 3.6 mM triethylamine] in a combination of

55:45 v/v. Before using, the mobile phase was degassed by passing it through a 0.45 μm filter and then pumped at an isocratic flow rate of 1 mL/min. Detection was accomplished by UV absorption at 280 nm.

2.3.12. Data analysis:

2.3.12.1. Fitting procedure:

All data were expressed as mean±SD unless otherwise indicated. To determine the kinetic constants for DEA formation and BPA consumption by liver and intestinal microsomal preparations, Michaelis-Menten models for single and two enzymes were fitted to DEA formation and BPA consumption rates using the Solver routine program in Microsoft Excel. The total sum of squares and Akiake information criteria were used to judge the goodness of fit, to guide in model selection [151].

For liver microsomes of the control and high caloric diet fed rats incubated with AM, a single enzyme model was used and the intrinsic clearance (CL_{int}) for DEA formation was calculated by determining the quotient of V_{max} to K_m. This model uses the following equation:

$$\boldsymbol{V = \frac{V_{max} \times [AM]}{k_m + [AM]}}$$

Where V is the rate of DEA formation, Vmax is the maximal rate of DEA formation, K_m is the affinity constant, [AM] is AM concentration.

JCR rats incubated with AM and BPA as well as the intestinal microsomes incubated with BPA, the same equation was used.

For intestinal microsomes incubated with AM, a two enzyme model was used. This model consists of a single saturable and a second linear component. The intrinsic clearance (CL_{int1}) for DEA formation was calculated by determining the quotient of Vmax to Km while CL_{int2} obtained from the Solver program.

This model uses the following equation:

$$\boldsymbol{V = \frac{V_{max1} \times [AM]}{K_{m1} + [AM]} + CL_{int2} \times [AM]}$$

Where K_{m1} and V_{max1} are the kinetic constants for high affinity enzyme, and CL_{int2} represents the V_{max} / K_m ratio for the low affinity enzyme.

For liver microsomes of the control and high caloric diet fed rats incubated with BPA, Michaelis-Menten equation using shape factor was the best fit to the resulted data and the CL_{int} was calculated by determining the quotient of V_{max} to K_m.

This model uses the following equation:

$$\boldsymbol{V = \frac{V_{max} \times [BPA]^n}{[K_m]^n + [BPA]^n}}$$

Where V is the rate of BPA consumption and n is the shape factor required to fit sigmoidal shapes.

2.3.12.2. Statistics:

One-way analysis of variance, Duncan's multiple range post hoc test and Student's unpaired *t* tests were used as appropriate to assess the significance of differences between groups.

Microsoft Excel (Microsoft, Redmond, WA), or SigmaPlot 11.0 (Systat software, Inc. Chicago, IL) were used in statistical analysis of data. The level of significance was set at $p < 0.05$.

Chapter3: Results

3.1. Incubation of liver microsomes with AM for control and high caloric diet fed rats:

The microsomes were obtained from the high caloric diet fed rats that are confirmed to be obese by significant increase in the body weight, caloric intake, and some biochemical plasma biomarker such as cholesterol, triglycerides and leptin compared to control rats that fed only on standard rat chow with normal drinking water [143]. Upon the incubation of (5- 155μM) of AM for 10 min with 1 mg/mL of microsomes, DEA formation was calculated for the control and obese groups (Figure 3-6). Under these conditions of time and protein concentration, the increases in DEA formation rates were found to be linear based on preliminary linearity studies[146]. Reduction of DEA formation was found in all obese groups compared to control (Figure 7). In addition, fitting of the data to the Michaelis-Menten equation was performed to determine the kinetic parameters (table 2). There was a significant reduction in V_{max} in HFD fed rats and significant increase in K_m in HFCS fed group compared to all other groups. Moreover, a significant reduction in Cl_{int} in all groups compared to control was observed.

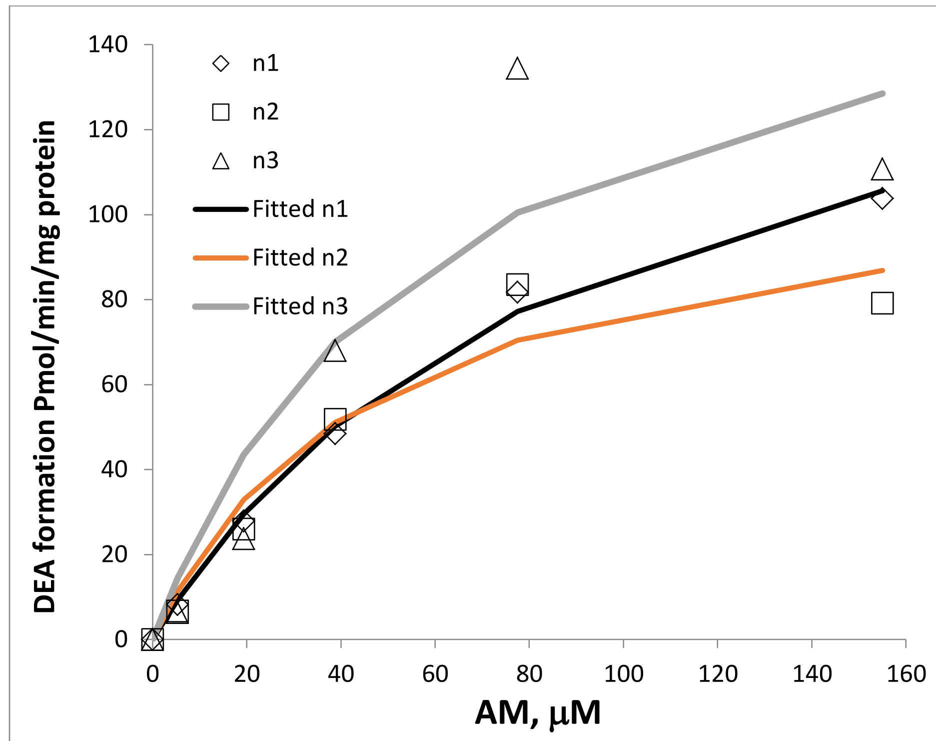

Figure 3: DEA formation rates from AM in hepatic microsomes of control rats. n1, n2, and n3 represent different microsomal preparation from different rats and each n is a separate run for the experiment. The fitted n1, n2, and n3 represent the predicted data for each n obtained by the best-fit model of the tested non-linear pharmacokinetics models

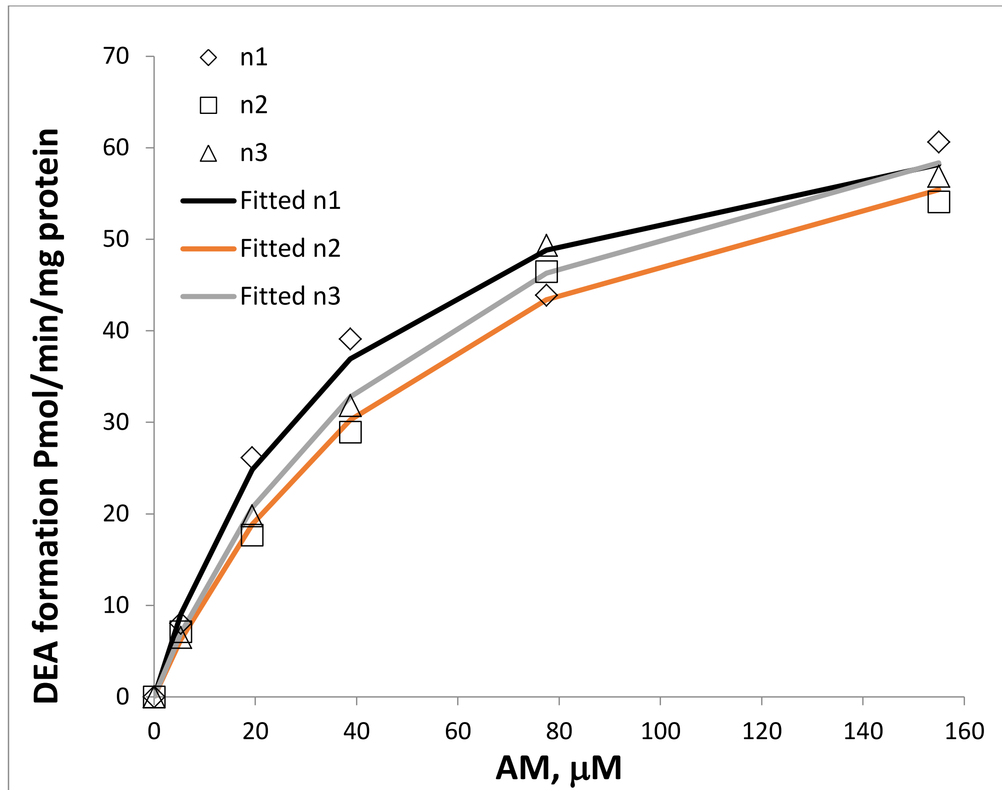

Figure 4: DEA formation rates from AM in hepatic microsmes of HFD fed rats. n1, n2, and n3 represent different microsomal preparation from different rats and each n is a separate run for the experiment. The fitted n1, n2, and n3 represent the predicted data for each n obtained by the best-fit model of the tested non-linear pharmacokinetics models

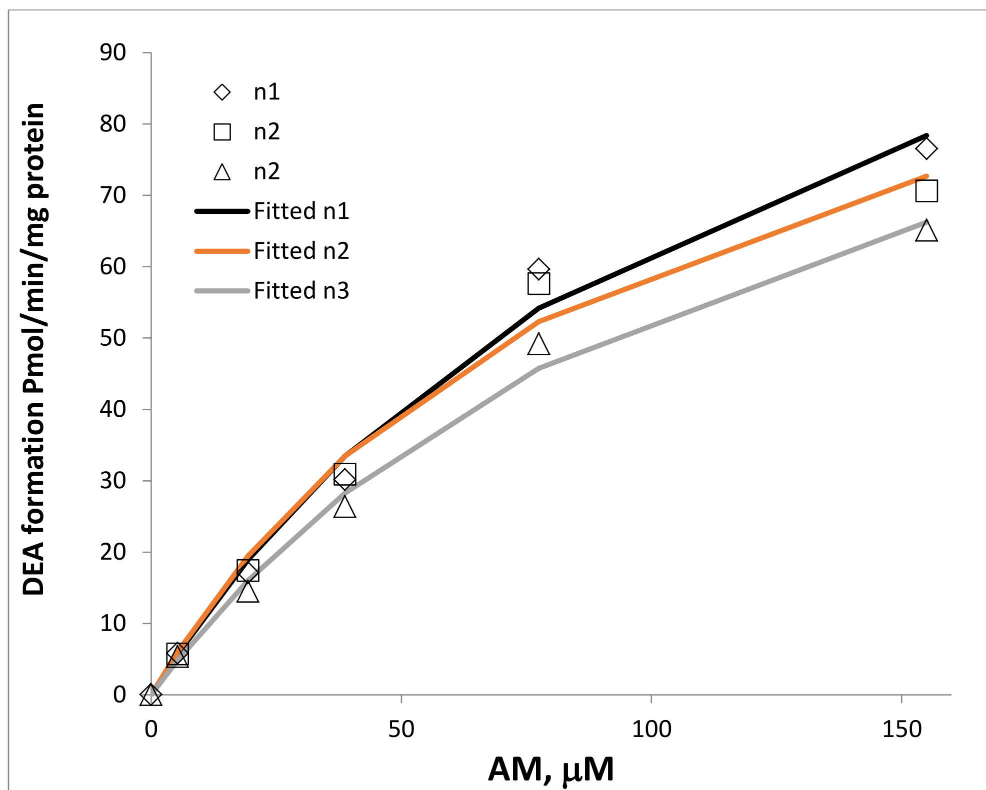

Figure 5: DEA formation rates from AM in hepatic microsomes of HFCS fed rats. n1, n2, and n3 represent different microsomal preparation from different rats and each n is a separate run for the experiment. The fitted n1, n2, and n3 represent the predicted data for each n obtained by the best-fit model of the tested non-linear pharmacokinetics models

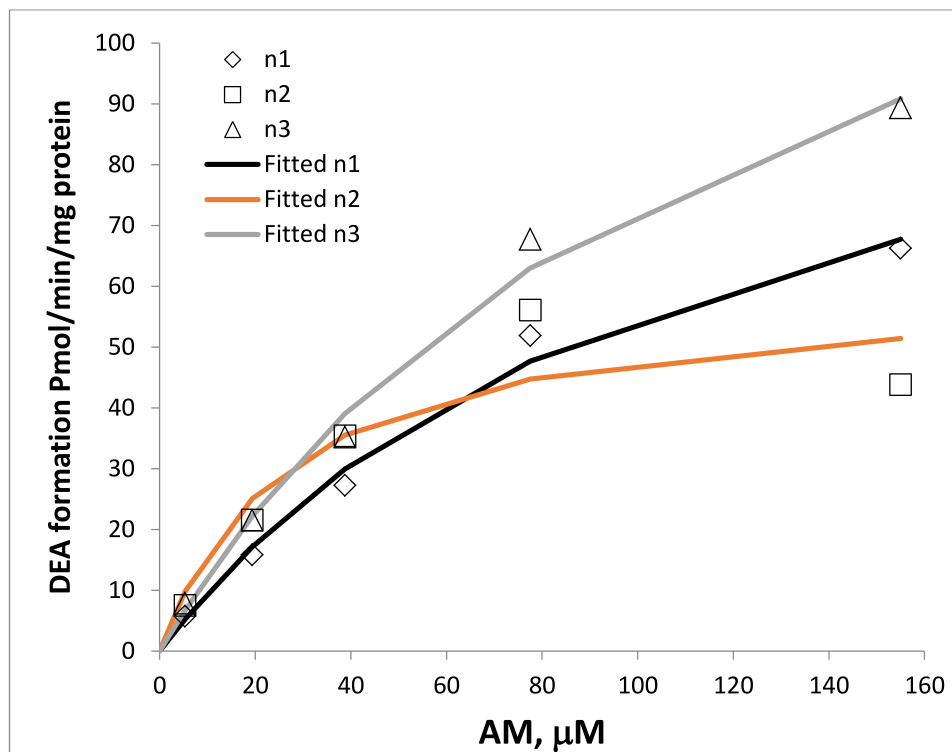

Figure 6: DEA formation rates from AM in hepatic microsomes of HFD-HFCS fed rats. n1, n2, and n3 represent different microsomal preparation from different rats and each n is a separate run for the experiment. The fitted n1, n2, and n3 represent the predicted data for each n obtained by the best-fit model of the tested non-linear pharmacokinetics models

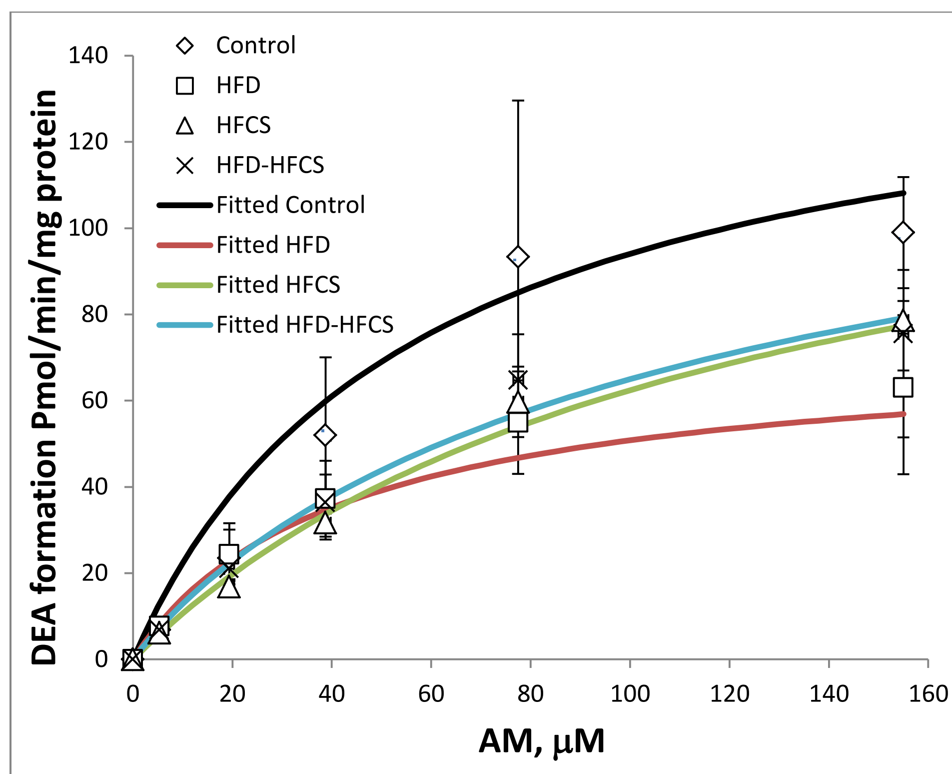

Figure 7: DEA formation rates (mean±SD, n=3) from AM in hepatic microsomes of control and obese rats. 1 mg/ml of protein was incubated with 5- 155µM of AM for 10 min. DEA formation rates were calculated using HPLC. The simple Michaelis-Menten model was fitted to the data (lines).

Table 2: Kinetic constants (mean±SD) for hepatic microsomal DEA formation in control and treated rats. Comparisons were done using one-way analysis of variance followed by Duncan's multiple range post hoc tests.[a] different from control, HFCS, and HFD-HFCS ($p<0.05$) [b] different from control and HFD ($p<0.05$) [c] different from HFD ($p<0.05$) [d] Different from all groups.

Parameter	Control	HFD	HFCS	HFD-HFCS
V_{max}, pmol/min/mg protein	150.07±28.74	73.13±6.10[a]	136.57±21.92	135.89±61.40
K_m, µM	59.76±21.57	44.28±15.34	118.54±12.92[b]	100.93±50.52[c]
Cl_{int} µL/min/mg protein	2.65±0.67[d]	1.78±0.52	1.15±0.15	1.50±0.50

3.2. Incubation of intestinal microsomal protein with AM for control and high caloric diet fed rats:

The two-enzyme system equation was the best fit to calculate the kinetic parameters of DEA formation (Figure 8-12). There were no significant changes in intestinal V_{max}, Cl_{int1}, and Cl_{int2} in all groups compared to control except for the HFCS fed group which had higher Cl_{int1} than control and HFD-HFCS fed rats. Regarding K_m, while HFCS fed group had significantly lower K_m value than control, the HFD-HFCS fed rats had significantly higher K_m value than all groups (table 3).

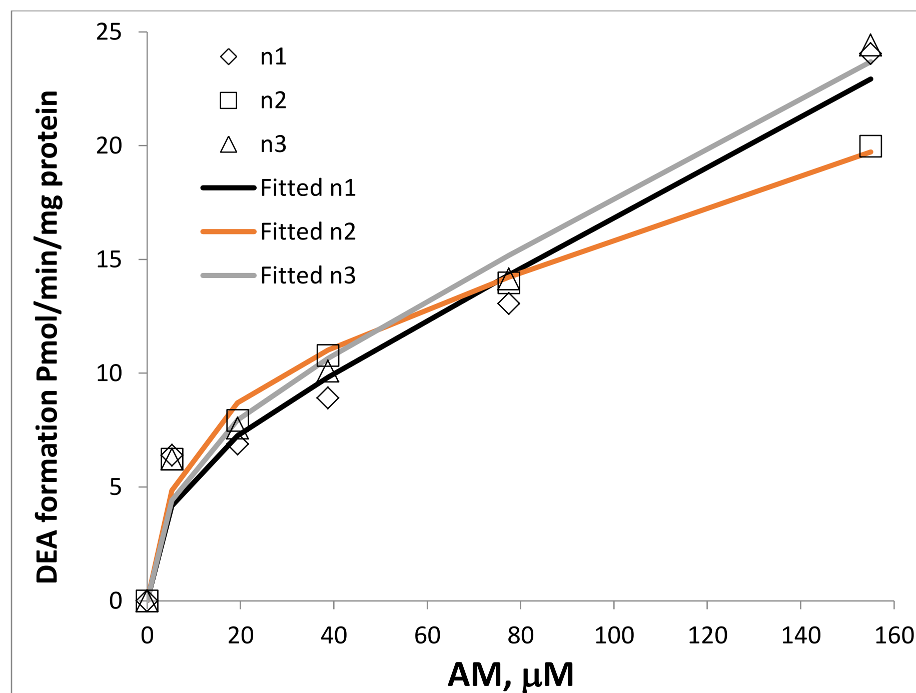

Figure 8: DEA formation rates from AM in intestinal microsomes of control rats. n1, n2, and n3 represent different microsomal preparation from different rats and each n is a separate run for the experiment. The fitted n1, n2, and n3 represent the predicted data for each n obtained by the best-fit model of the tested non-linear pharmacokinetics models

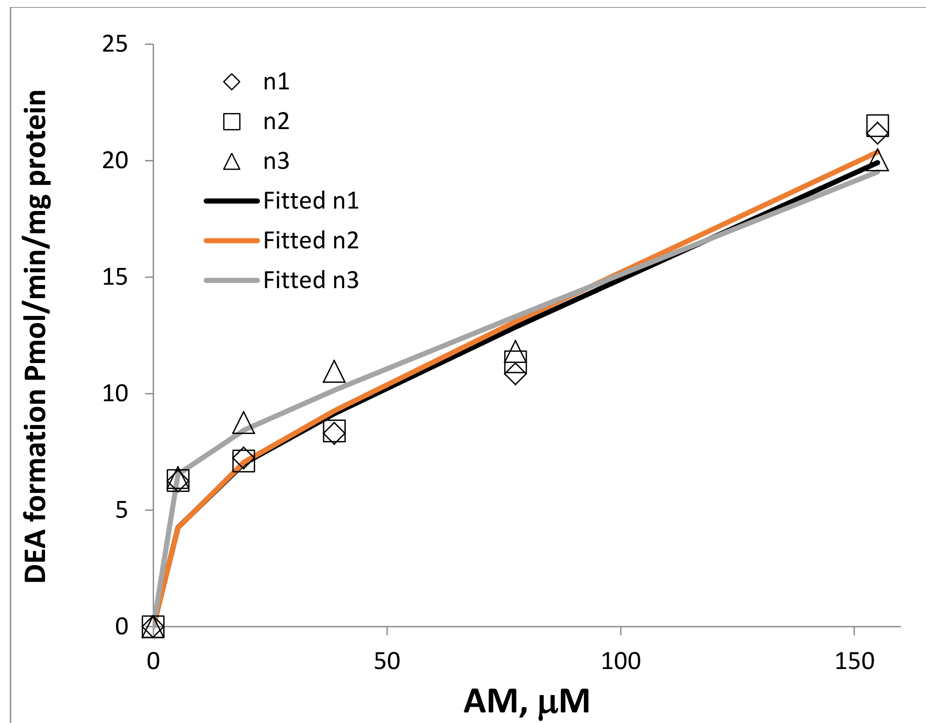

Figure 9: DEA formation rates from AM in intestinal microsomes of HFD fed rats. n1, n2, and n3 represent different microsomal preparation from different rats and each n is a separate run for the experiment. The fitted n1, n2, and n3 represent the predicted data for each n obtained by the best-fit model of the tested non-linear pharmacokinetics models

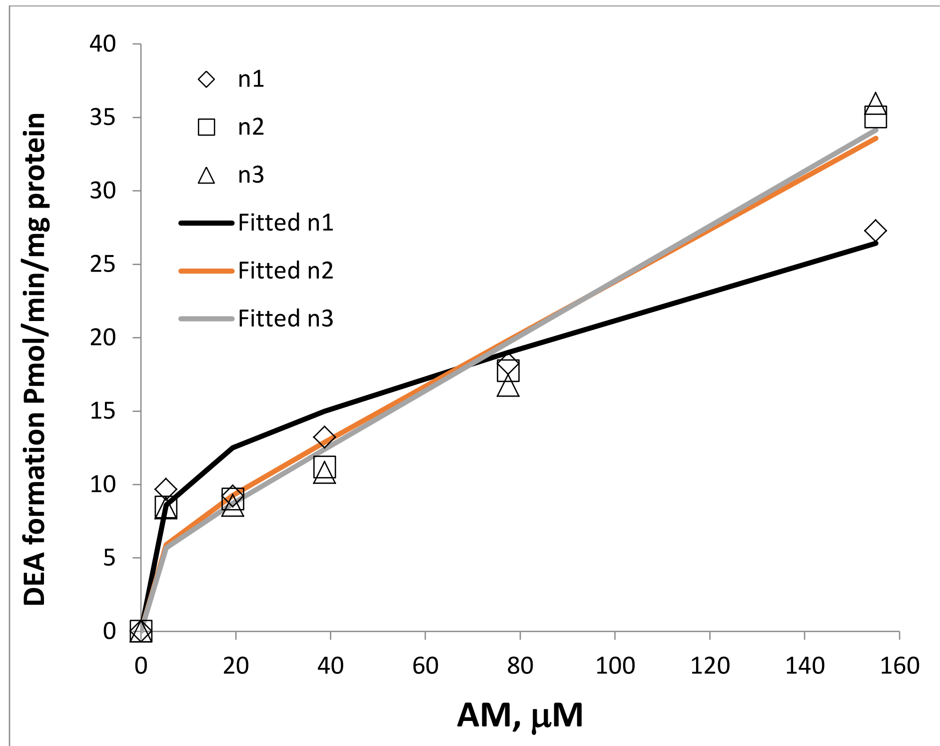

Figure 10: DEA formation rates from AM in intestinal microsomes of HFCS fed rats. n1, n2, and n3 represent different microsomal preparation from different rats and each n is a separate run for the experiment. The fitted n1, n2, and n3 represent the predicted data for each n obtained by the best-fit model of the tested non-linear pharmacokinetics models

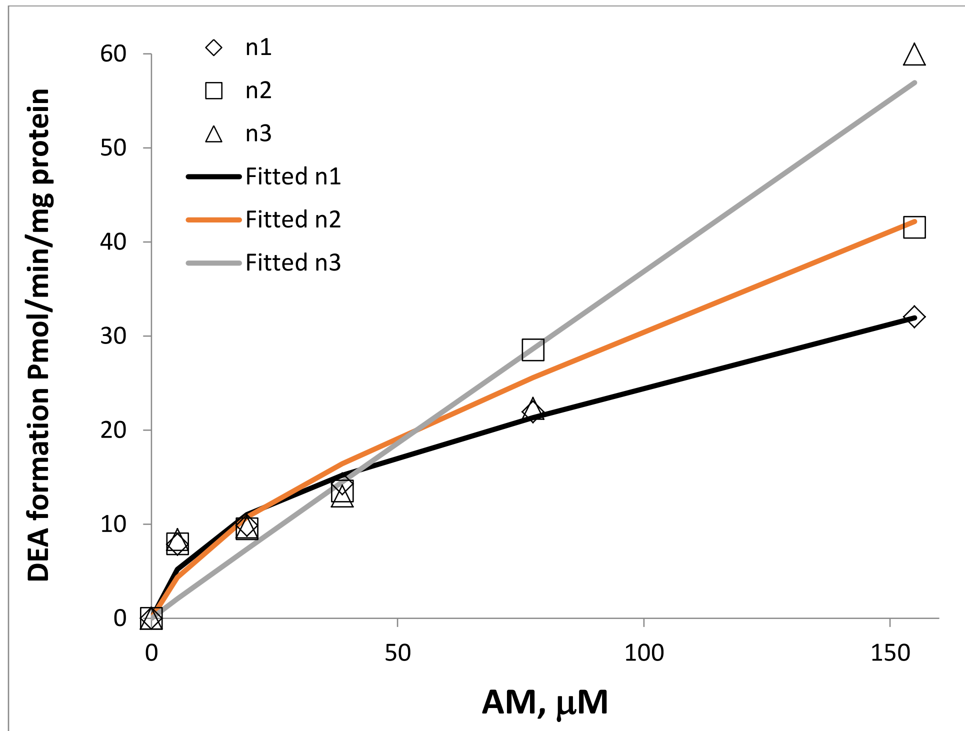

Figure 11: DEA formation rates from AM in intestinal microsomes of HFD-HFCS fed rats. n1, n2, and n3 represent different microsomal preparation from different rats and each n is a separate run for the experiment. The fitted n1, n2, and n3 represent the predicted data for each n obtained by the best-fit model of the tested non-linear pharmacokinetics models

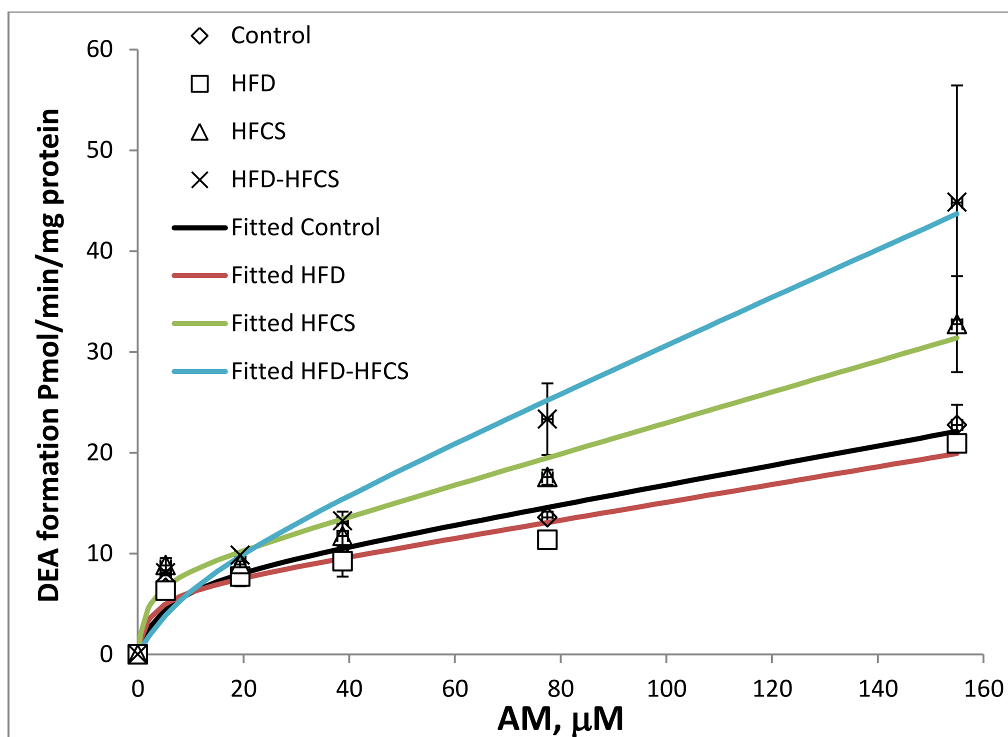

Figure 12: DEA formation rates (mean±SD, n=3) from AM in intestinal microsomes of control and obese rats. 1 mg/ml of protein was incubated with 5- 155µM of AM for 10 min. DEA formation rates were calculated using HPLC. The two-enzyme model was fitted to the data (lines).

Table 3: Kinetic constants (mean±SD) for intestinal microsomes DEA formation in control and treated rats. Comparisons were done using one-way analysis of variance followed by Duncan's multiple range post hoc tests.[a] different from control ($p<0.05$) [b] different from all groups ($p<0.05$) [c] different from control and HFCS ($p<0.05$).

Parameter	Control	HFD	HFCS	HFD-HFCS
V_{max}, pmol/min/mg protein	7.74±1.86	6.53±0.61	7.89±3.73	8.22±6.77
K_m, µM	4.93±1.24	2.58±1.38	1.55±1.02[a]	10.33±2.55[b]
Cl_{int1} µL/min/mg protein	1.57±0.05	3.67±3.19	5.77±2.05[c]	0.75±0.65
Cl_{int2} µL/min/mg protein	0.09±0.02	0.08±0.006	0.15±0.05	0.23±0.12

3.3. Incubation of liver microsomal protein with AM for lean and obese JCR rats:

The microsomes obtained from the liver of both lean and genetically obese rats. The genetically obese rats had significant increase in body weight and biochemical plasma markers such as cholesterol, triglycerides, LDL, HDL, glucose and insulin compared to their lean. The isolated rat microsomes of the liver of both groups were incubated with AM for 10 min and DEA formation rates were plotted against drug concentration (Figure 13-15). Simple Michaelis-Menton equation was used to calculate the kinetic parameters for DEA formation in both groups. There were no significant changes in V_{max} between both groups but a significant reduction in the K_m value for the obese group was obtained indicating higher affinity for the metabolizing enzymes in the obese group. As a result, the Cl_{int} was higher in the obese group compared to the lean control rats (table 4).

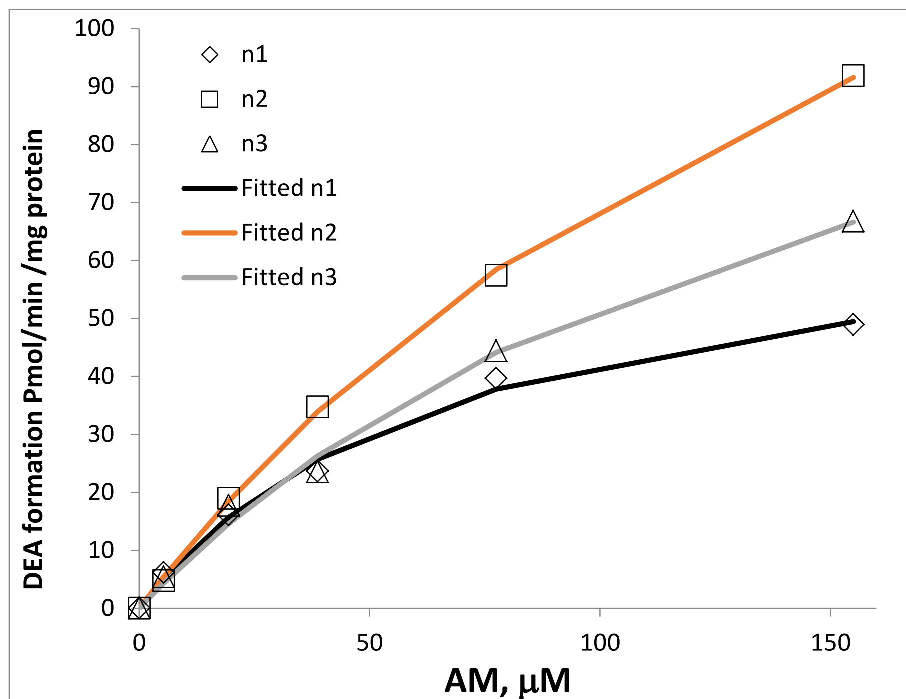

Figure 13: DEA formation rates from AM in hepatic microsomes of lean JCR rats. n1, n2, and n3 represent different microsomal preparation from different rats and each n is a separate run for the experiment. The fitted n1, n2, and n3 represent the predicted data for each n obtained by the best-fit model of the tested non-linear pharmacokinetics models

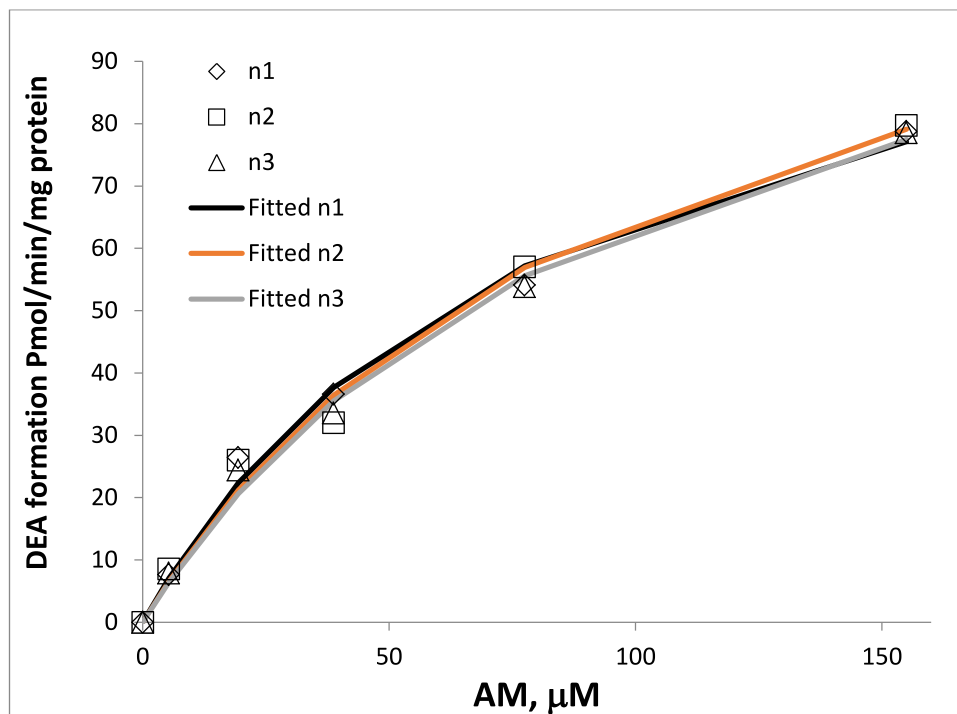

Figure 14: DEA formation rates from AM in hepatic microsomes of obese JCR rats. n1, n2, and n3 represent different microsomal preparation from different rats and each n is a separate run for the experiment. The fitted n1, n2, and n3 represent the predicted data for each n obtained by the best-fit model of the tested non-linear pharmacokinetics models

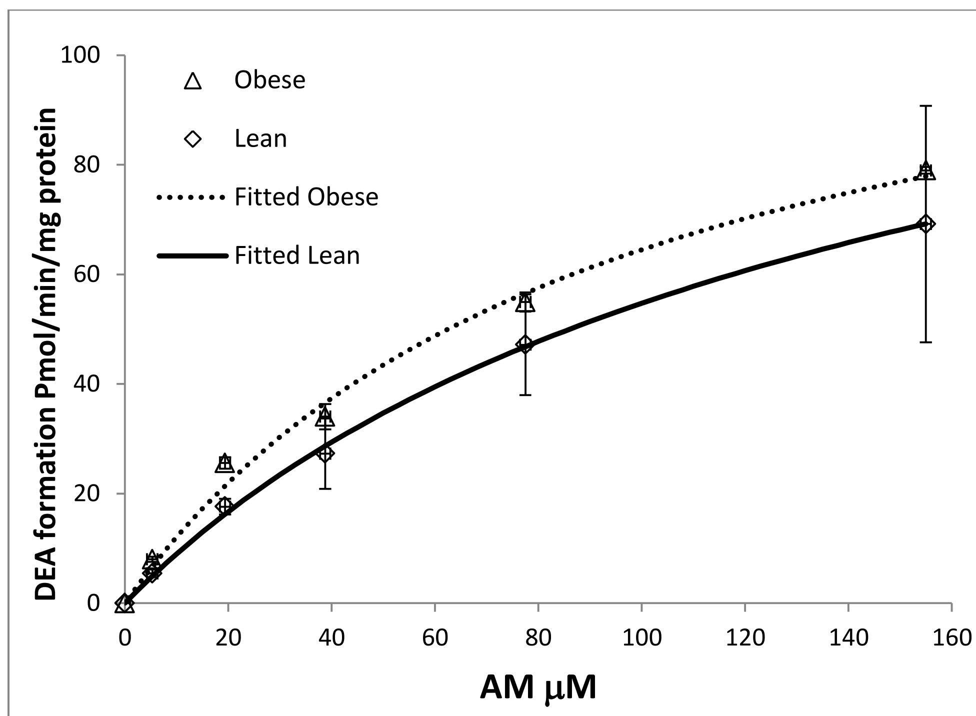

Figure 15: DEA formation rates (mean±SD, n=3) from AM in hepatic microsomes of lean and obese JCR rats. 1 mg/ml of protein was incubated with 5- 155µM of AM for 10 min. DEA formation rates were calculated using HPLC. The simple Michaelis-Menten model was fitted to the data (lines).

Table 4: Kinetic constants (mean±SD) for hepatic microsomal DEA formation in lean and obese rats. Comparisons were done using student's unpaired t-test. [a,b] different from lean ($p<0.05$).

Parameter	Lean	Obese
V_{max}, pmol/min/mg protein	139.50±69.97	125.30±5.99
K_m, µM	144.11±68.43	94.24±9.67[a]
Cl_{int} µL/min/mg protein	0.97±0.11	1.33±0.08[b]

3.4. Incubation of liver microsomal protein with BPA for control and high caloric diet fed rats:

The consumption of BPA by the microsomes was calculated by measuring the difference between incubation of the drug with the microsomes at zero time (no metabolism) and at 10 minutes after incubation (time required for the metabolism). Then the consumption rates were plotted against the BPA concentrations (Figure 16-20). Regarding the kinetic constants, the Michaelis-Menton equation using shape factor was best fitted to calculate the PK parameters (table 5). Although the control group exhibited higher V_{max} value than the obese groups, all obese groups that fed on high caloric diets exhibited lower K_m values and significant increase in Cl_{int} compared to control group.

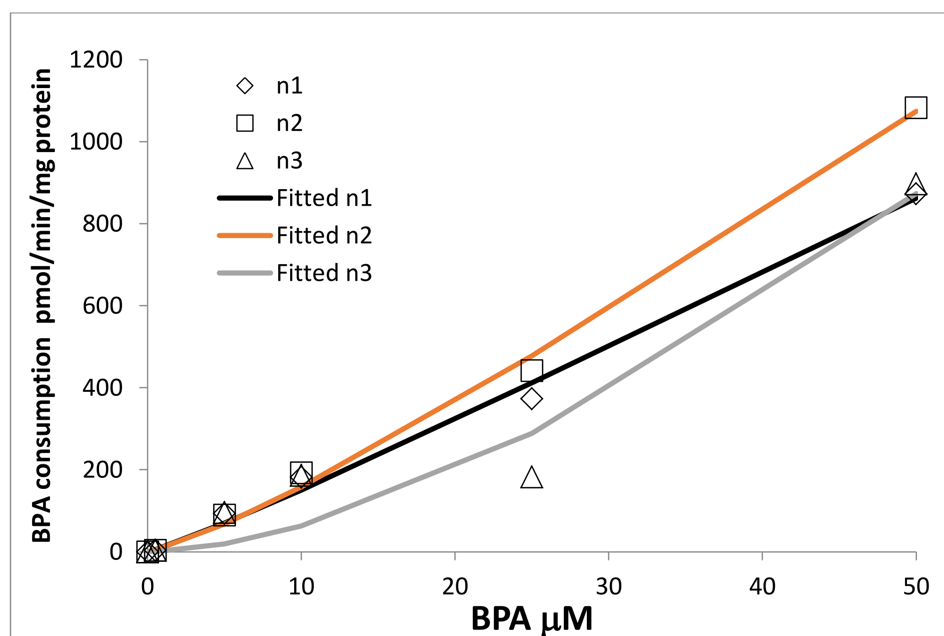

Figure 16: BPA consumption rates in hepatic microsomes of control rats. n1, n2, and n3 represent different microsomal preparation from different rats and each n is a separate run for the experiment. The fitted n1, n2, and n3 represent the predicted data for each n obtained by the best-fit model of the tested non-linear pharmacokinetics models

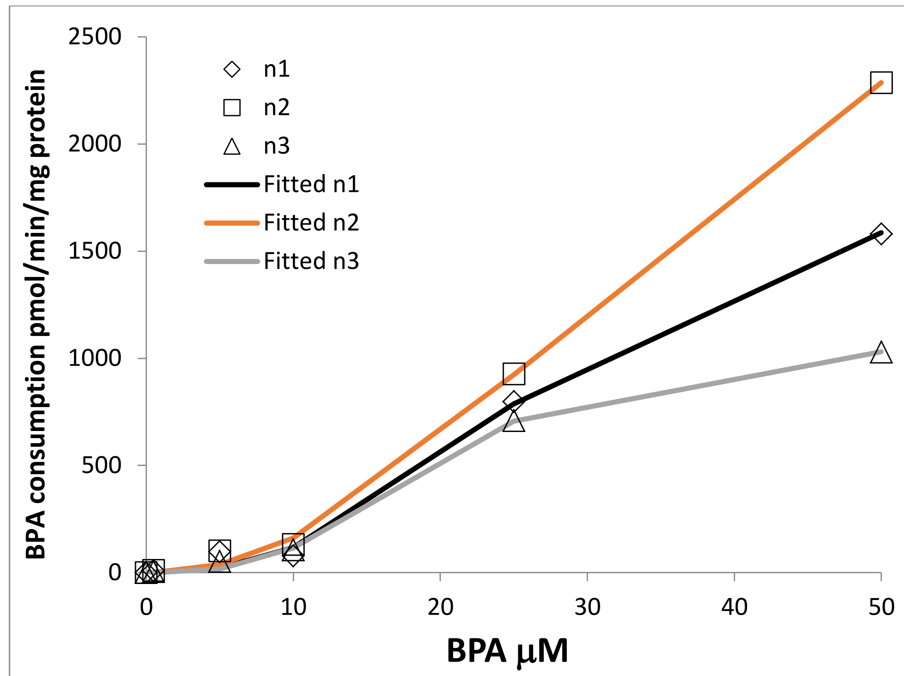

Figure 17: BPA consumption rates in hepatic microsomes of HFD fed rats. n1, n2, and n3 represent different microsomal preparation from different rats and each n is a separate run for the experiment. The fitted n1, n2, and n3 represent the predicted data for each n obtained by the best-fit model of the tested non-linear pharmacokinetics models

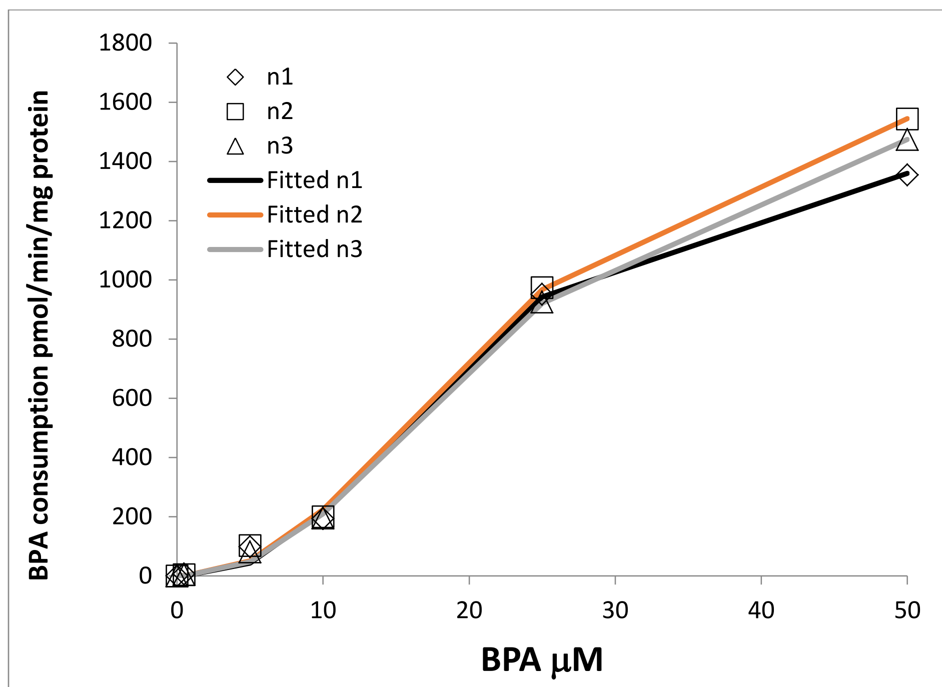

Figure 18: BPA consumption rates in hepatic microsomes of HFCS fed rats. n1, n2, and n3 represent different microsomal preparation from different rats and each n is a separate run for the experiment. The fitted n1, n2, and n3 represent the predicted data for each n obtained by the best-fit model of the tested non-linear pharmacokinetics models

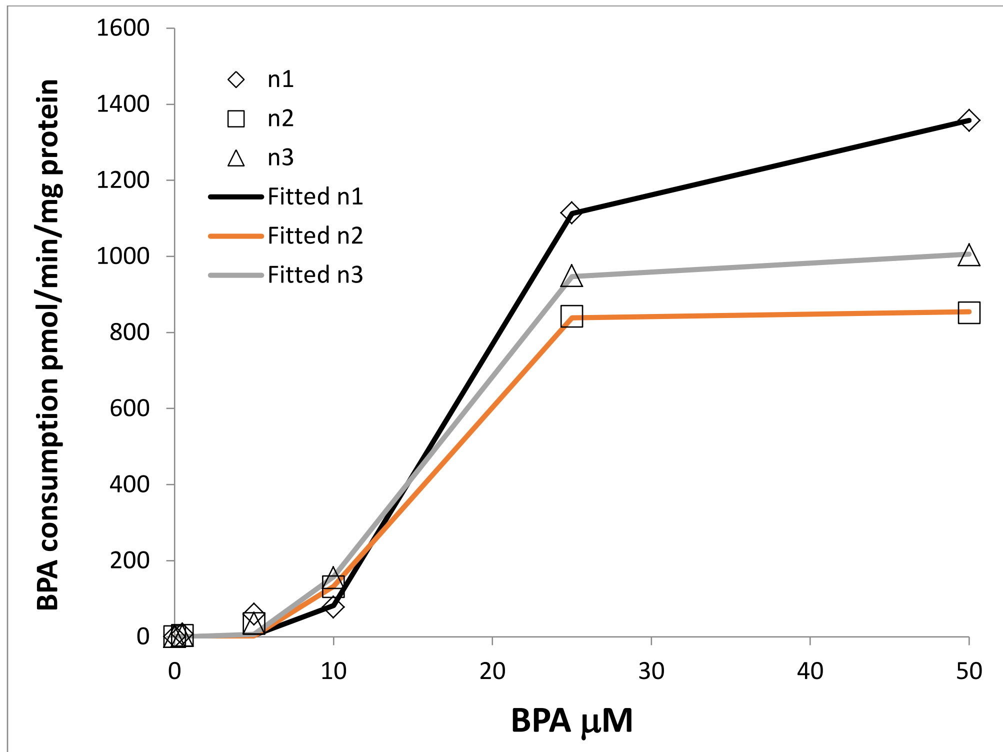

Figure 19: BPA consumption rates in hepatic microsomes of HFD-HFCS rats. n1, n2, and n3 represent different microsomal preparation from different rats and each n is a separate run for the experiment. The fitted n1, n2, and n3 represent the predicted data for each n obtained by the best-fit model of the tested non-linear pharmacokinetics models

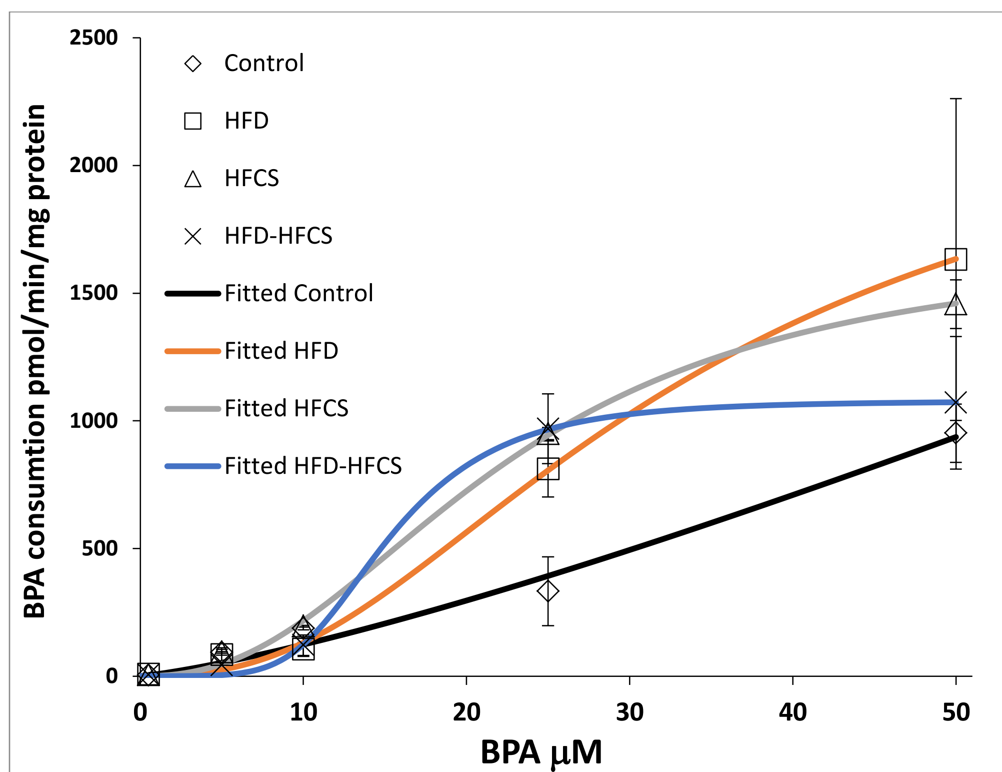

Figure 20: BPA consumption rates (mean±SD, n=3) in hepatic microsomes of control and obese rats. 1 mg/ml of protein was incubated with 0.5- 50μM of BPA for 10 min. BPA consumption rates were calculated using HPLC. The sigmoidal model was fitted to the data (lines)

Table 5: Kinetic constants (mean±SD) for hepatic microsomal BPA consumption in control and obese rats. Comparisons were done using one-way analysis of variance followed by Duncan's multiple range post hoc tests. a different from each of the high calorie groups ($p<0.05$).

Parameter	Control	HFD	HFCS	HFD-HFCS
V_{max}, nmol/min/mg protein	12.4± 2.54[a]	2.37± 1.49	1.69± 0.16	1.07±2.65
K_m, µM	367.23±153.98[a]	31.32±11.73	22.62±2.007	15.18±2.65
Cl_{int} µL/min/mg protein	37.20±12.55[a]	70.89±19.14	74.75±1.95	70.43±5.25

3.5. Confirmation of glucuronidation process by β-glucuronidase enzyme:

To determine whether BPA is degraded mainly by glucuronidation or by any other process, β-glucuronidase enzyme was added to the incubation mixture to inverse the reaction of UGTs. Then the concentration of BPA after 6hr of adding the hydrolyzing enzyme was compared to the BPA concentration at 0 time and at 10 min which is the time of metabolism reaction. We found that the BPA concentration after 6 hr of adding the β-glucuronidase enzyme is around 75% the concentration of BPA at 0 time indicating the metabolism of BPA to BPA-glucuronide (Figure21).

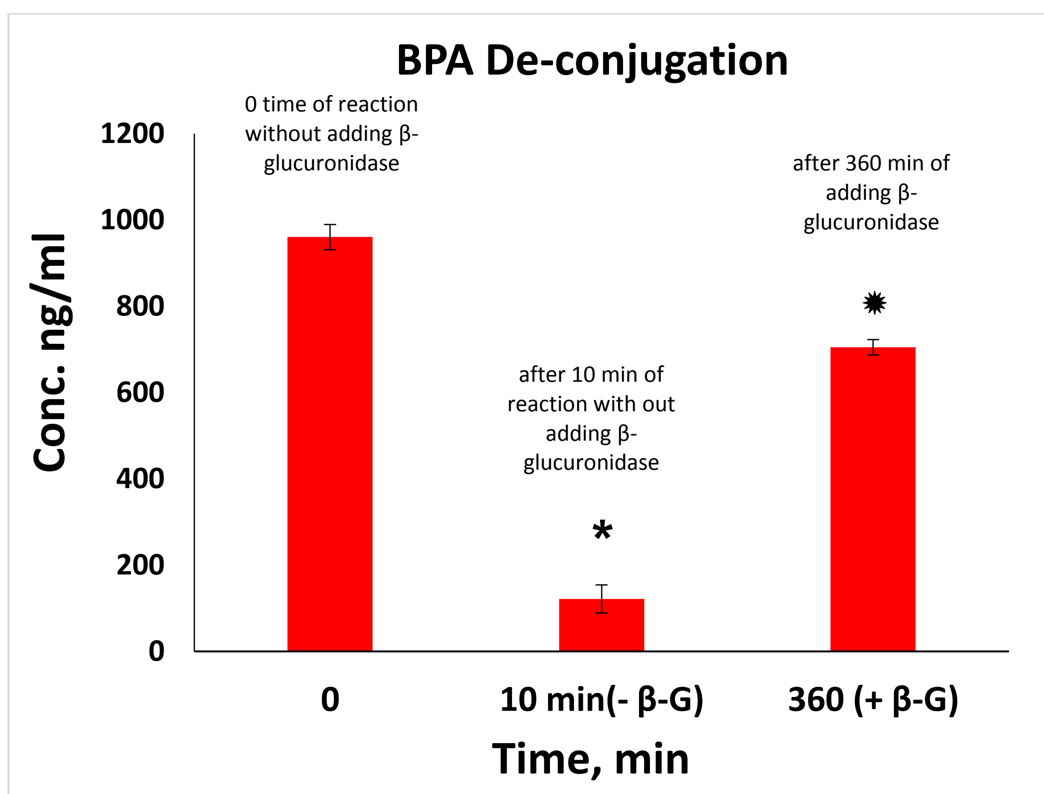

Figure 21: BPA concentration (mean±SD) at 0, 10, and 360 min of metabolism and hydrolysis reactions in liver microsomes of control rats. Comparisons were done using one-way analysis of variance followed by Duncan's multiple range post hoc tests. * different from 0 time and 360 min ($p<0.05$). ✷ different from 0 time and 10 min ($p<0.05$).

3.6. Incubation of intestinal microsomal protein with BPA for control and high caloric diet fed rats:

Similar to the hepatic microsomes, the consumption rates were plotted against the BPA concentrations for control and treated microsomes (Figure 22-25). There was a trend toward an increase in the consumption of BPA in HFD and HFCS fed rats comparing to control rats (Figure 26). Using the Michaelis-Menton equation to calculate the kinetic constants for the reaction, there was a significant increase in Cl_{int} in HFD and HFCS fed groups compared to control with no significant changes in V_{max} and K_m values between groups (Table 6).

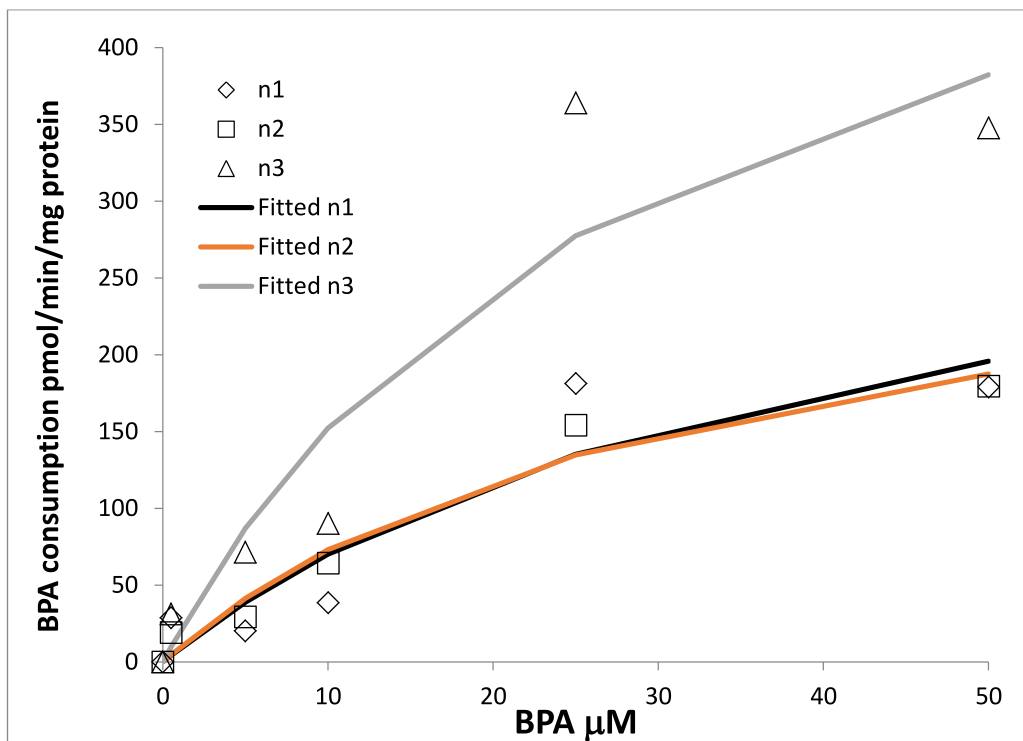

Figure 22: BPA consumption rate in intestinal microsomes of control rats. n1, n2, and n3 represent different microsomal preparation from different rats and each n is a separate run for the experiment. The fitted n1, n2, and n3 represent the predicted data for each n obtained by the best-fit model of the tested non-linear pharmacokinetics models

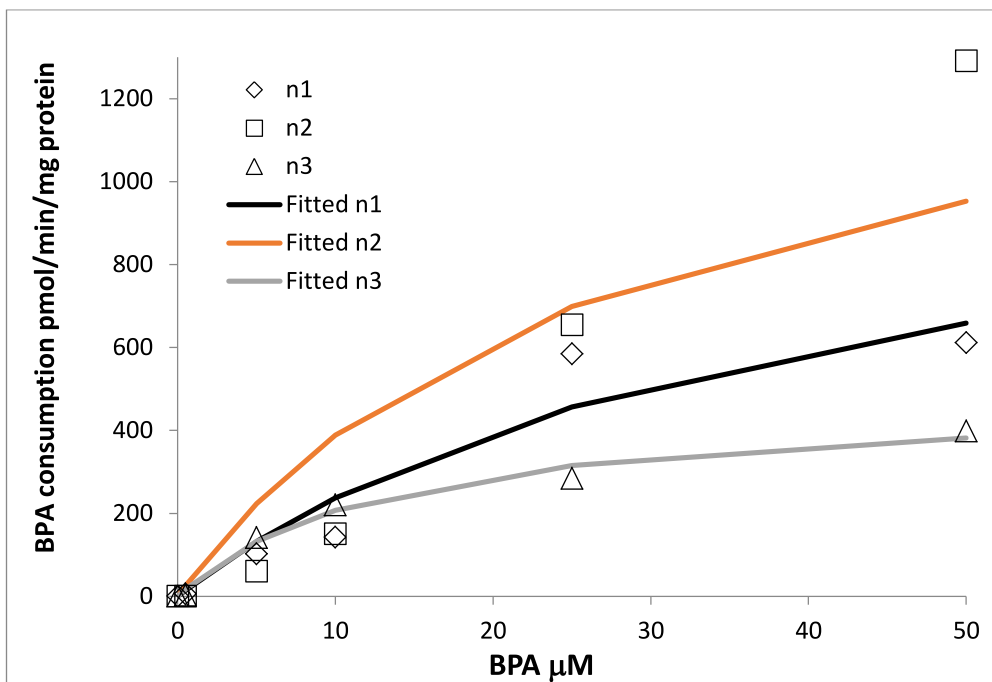

Figure 23: BPA consumption rate in intestinal microsomes of HFD fed rats. n1, n2, and n3 represent different microsomal preparation from different rats and each n is a separate run for the experiment. The fitted n1, n2, and n3 represent the predicted data for each n obtained by the best-fit model of the tested non-linear pharmacokinetics models

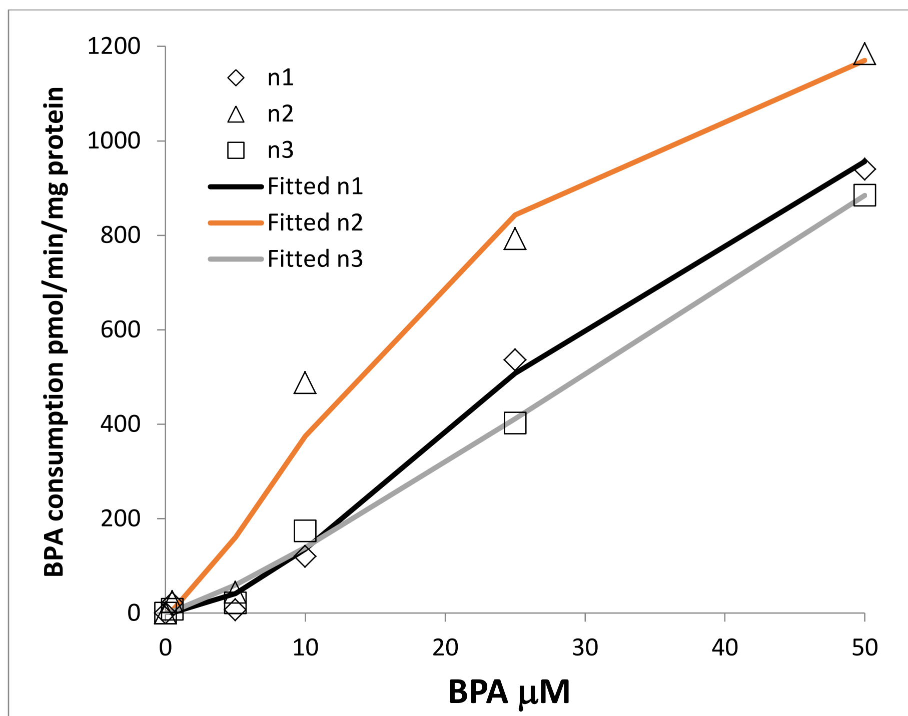

Figure 24: BPA consumption rate in intestinal microsomes of HFCS fed rats. n1, n2, and n3 represent different microsomal preparation from different rats and each n is a separate run for the experiment. The fitted n1, n2, and n3 represent the predicted data for each n obtained by the best-fit model of the tested non-linear pharmacokinetics models

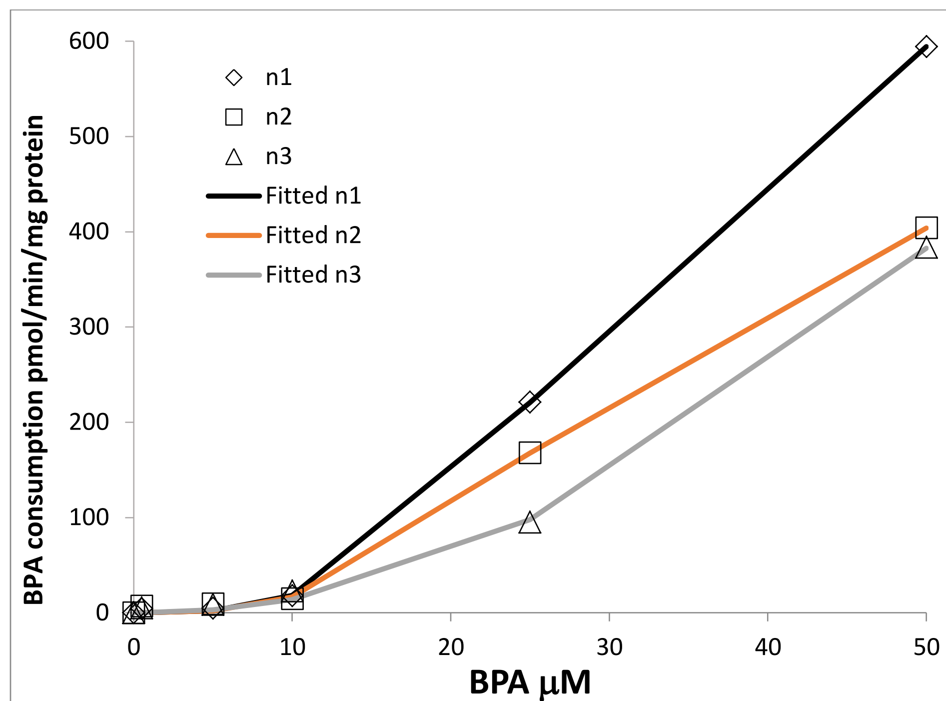

Figure 25: BPA consumption rate in intestinal microsomes of HFD-HFCS fed rats. n1, n2, and n3 represent different microsomal preparation from different rats and each n is a separate run for the experiment. The fitted n1, n2, and n3 represent the predicted data for each n obtained by the best-fit model of the tested non-linear pharmacokinetics models

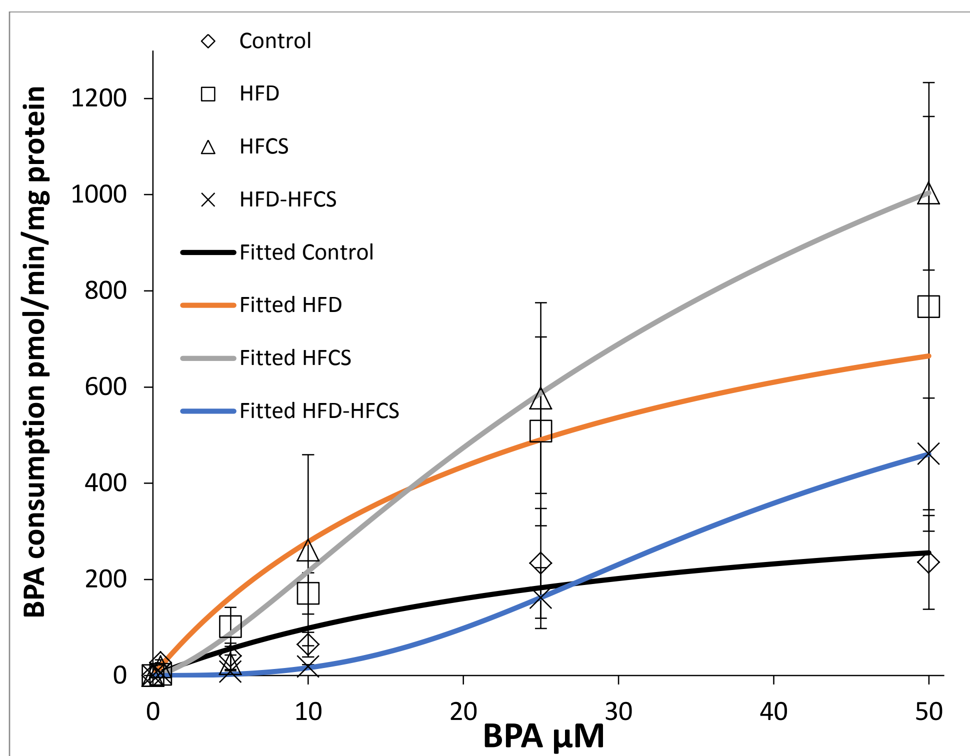

Figure 26: BPA consumption rates (mean±SD) in intestinal microsomes of control and obese rats. 1 mg/ml of protein was incubated with 0.5- 50µM of BPA for 10 min. BPA consumption rates were calculated using HPLC. The sigmoidal model was fitted to the control and HFD groups data whereas the simple Michaelis-Menten model was fitted to the HFCS and HFD-HFCS fed groups data (lines)

Table 6: Kinetic constants (mean±SD) for intestinal microsomal BPA consumption in control and obese rats. Comparisons were done using one-way analysis of variance followed by Duncan's multiple range post hoc tests. [a] different from HFD and HFCS ($p<0.05$) [b] different HFCS ($p<0.05$).

Parameter	Control	HFD	HFCS	HFD-HFCS
V_{max}, nmol/min/mg protein	0.42±0.16	1.05±0.52	2.91±2.47	1.15±0.88
K_m, µM	34.28±5.44	27.15±13.31	85.43±98.68	55.54±39.28
Cl_{int} µL/min/mg protein	12.88±6.42[a]	39.59±11.68	47.11±21.67	20.09±3.59[b]

3.7. Incubation of liver microsomal protein with BPA for lean and obese JCR:

No changes in the consumption rate of BPA between both groups were noticed as shown in (Figure 27-29). The simple Michaelis-Menten equation was used to calculate the kinetic constants for the reactions (table 7). Comparisons using student's unpaired t-test showed that there is no significant differences between V_{max}, K_m, and Cl_{int} between both liver microsomes of lean and obese rats.

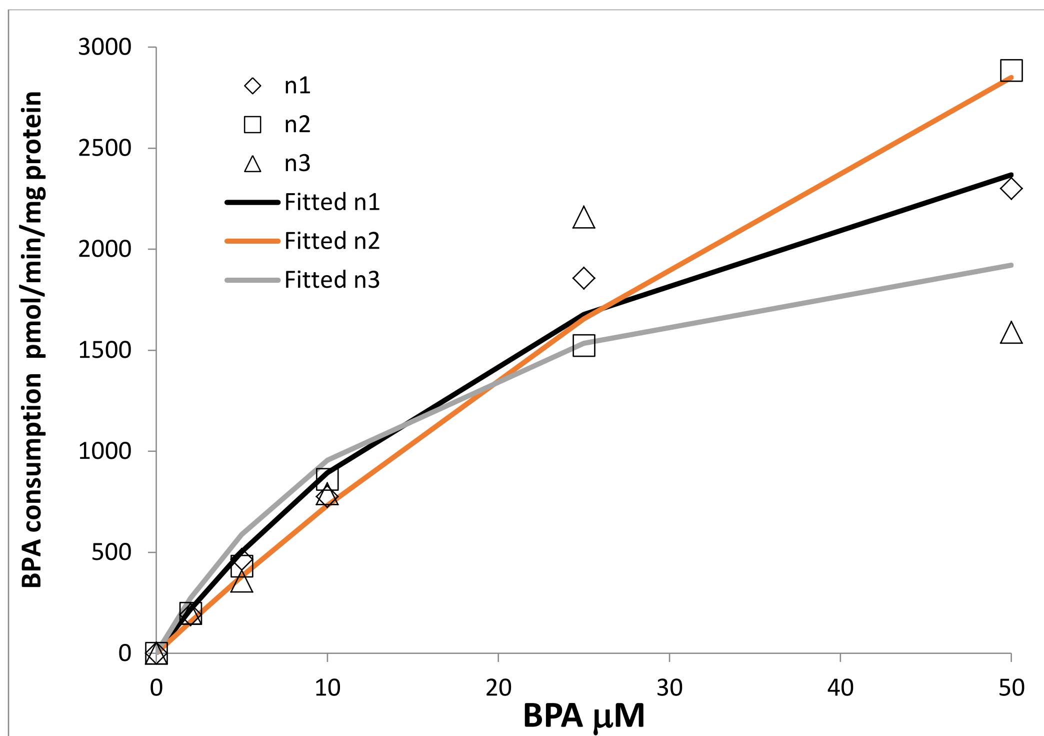

Figure 27: BPA consumption rates in hepatic microsomes of lean JCR rats. n1, n2, and n3 represent different microsomal preparation from different rats and each n is a separate run for the experiment. The fitted n1, n2, and n3 represent the predicted data for each n obtained by the best-fit model of the tested non-linear pharmacokinetics models

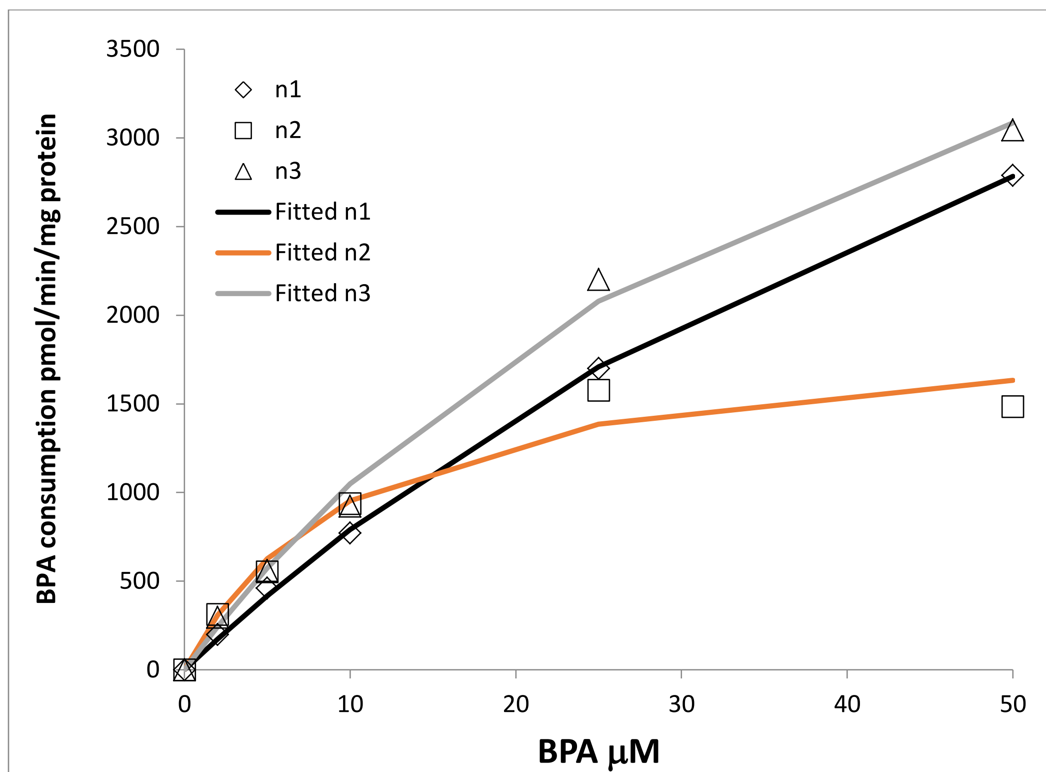

Figure 28: BPA consumption rates in hepatic microsomes of obese JCR rats. n1, n2, and n3 represent different microsomal preparation from different rats and each n is a separate run for the experiment. The fitted n1, n2, and n3 represent the predicted data for each n obtained by the best-fit model of the tested non-linear pharmacokinetics models

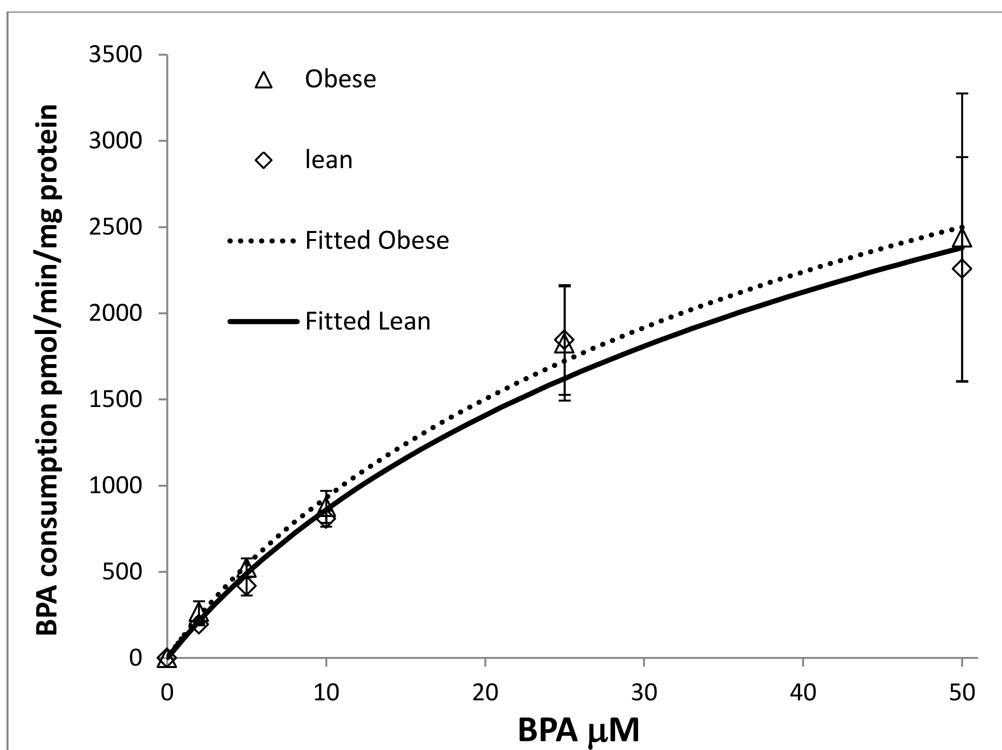

Figure 29: BPA consumption rates (mean±SD) in hepatic microsomes of lean and obese JCR rats. 1 mg/ml of protein was incubated with 0.5- 50μM of BPA for 10 min. BPA consumption rates were calculated using HPLC. The simple Michaelis-Menten model was fitted to the data (lines)

Table 7: Kinetic constants (mean±SD) for hepatic microsomal BPA consumption in lean and obese JCR rats. Comparisons were done using student's unpaired t-test. No statistical difference found between both groups.

Parameter	Lean	Obese
V_{max}, nmol/min/mg protein	5.61±4.06	5.15±2.85
K_m, μM	60.48±60.43	47.53±36.96
Cl_{int} μL/min/mg protein	115.40±36.71	133.13±47.81

Chapter 4: Discussion

Obesity can cause multiorgan dysfunction and multiple diseases that require pharmacotherapy intervention. Because of the physiological changes associated with obesity and the medications administered due to the associated comorbidities, obesity may cause changes to the pharmacokinetics of drugs and eventually affecting their efficacy and safety.

Several research studies investigated the impact of obesity on metabolism whether the impact on phase I or phase II metabolising enzymes. These studies mainly focused on the expression of mRNA or protein levels of the metabolizing enzymes. Here we are exploring the obesity impact on the functional activity of these enzymes using different substrates for each phase, different tissues, and different models of obesity using dietary induced obesity models or genetic models.

The obese animal models developed in our lab fed on a diet that mimics Western-lifestyle food that encompass the wide use of high fructose corn syrup and a food rich in fat. Aat the end of feeding, they had all gained weight significantly compared to the lean control group. With the importance of genomics and the diseases related to that, we did not neglect to the study the metabolism of our substrates in genetic obese model using JCR rats.

Regarding the use of our substrates, we used amiodarone as a substrate of phase I metabolism as it is mainly metabolised by CYPs enzymes in human and rats as well as its widely administered in obese patients having cardiovascular diseases. In addition,

we used BPA as a substrate of phase II metabolism as it is mainly metabolised by UGTs enzymes and it is widely distributed environmental pollutant and widely used in the industry in synthesis of polycarbonate plastics.

4.1. Fitting procedure:

Computerization is widely used in determination of PK parameters. There are several computerized fitting of models to a data set to assess the goodness of fit. Generalized reduced gradient algorithm was used as an iterative algorithm to arrive at solution based on least squares estimation when an initial value was used to get the required PK parameters. In addition, to avoid the convergence to wrong value, the process is repeated several times using different initial values.

To determine the enzyme kinetics for DEA formation and BPA consumption in liver and intestine of diet induced obese rats and genetically obese rat, several models were tested in each tissue and rat model with either AM or BPA. These include; simple Michaelis-Menton kinetics, sigmoidal Michaelis-Menton kinetics with the shape factor, two enzyme system, and Substrate Inhibition kinetics [152].

To assess goodness of fit for these models, Akaike Information criterion (AIC) was used. AIC is commonly used in clinical data analysis and pharmacokinetic analysis, as it is flexible and useful. AIC is based on maximum likehood estimation and it selects the model that minimizes mean squarerd error of prediction or estimation [153]. The

model with the smallest AIC value is considered to be the most appropriate model and fits the actual data [154].

$$AIC = N \ln R_e + 2\,p$$

Where N is the number of data points.

R_e is the residual sum of squares,

p is the number of parameters.

4.2. The effect of diet induced obesity on the metabolism of amiodarone in liver and intestine:

All high caloric diet fed groups after 14 weeks of feeding had a significant increase in body weight compared to control. Their weight was 802, 789, 719, and 639 g for HFD-HFCS, HFD, HFCS, and control groups respectively. In addition, starting from week 4 of treatment, all groups had a significant increase in caloric intake compared to control until the end of the study. Moreover, a significant gain in perinephric fat mass were observed in all high caloric diet groups compared to the control and the HFD fed group had the highest fat mass between all groups [143]. After confirmation of weight gain, our lab also studied some biochemical markers that are characteristic feature associated with obesity in the plasma of all groups. Both HFD and HFD-HFCS fed groups had a significant increase in cholesterol plasma levels compared to the control group while HFCS fed group showed a trend toward increase in plasma cholesterol levels. In addition, HFCS and HFD-HFCS fed groups had a significant increase in the mean plasma levels of triglyceride compared to the control group. Eventually our lab developed obese models characterised by hyperinsulinemia with a small elevation of plasma

glucose and without any significant changes in alanine aminotransferase and aspartate aminotransferase that are characteristics of liver injury. Hence, we omitted the effect of diabetes and liver injury on the impact of obesity on drug metabolizing enzymes.

Our finding demonstrated that the intrinsic clearance of amiodarone was decreased in the liver of all obese groups compared to control. AM is metabolized by several enzymes including; CYP3A4, 2C8, 1A2 and 2D6 in human and CYP3A1 and 1A1 in rats. Data from our lab showed a decreased expression in hepatic *CYP3A2* mRNA and protein levels in all high caloric diet groups as well as a decrease in hepatic *CYP3A1* protein expression in both HFD and HFCS/HFD groups [143].
Furthermore we found that hepatic CYP1A1had a small decrease in protein levels of HFD and HFCS/HFD without changes in mRNA levels. In addition, we found that the V_{max}, which corresponds to the capacity or the amount of the enzymes available for the reaction, was significantly decreased in HFD fed rats with a decreased trend in the other groups compared to control. Our findings are largely correlated to many previous studies in the literature on humans and rats where they found either a reduction in the metabolic activity or a reduction in the expression of mRNA or protein levels of CYP3A in diet induced obesity [64, 155]. Hyperlipidemia and inflammation associated with obesity can have effects on the drug metabolizing enzymes expression and activity. It has been reported previously in our lab that HL could influence expression of metabolizing enzymes and transporters in liver. We examined the effect of HL induced by poloxamer 407 in rat model on metabolism of amiodarone. Shayeganpour reported that CYP3A1, CYP3A2 and CYP2C11 in

hyperlipidemic rats compared to normolipidemic rats. In addition, Shayeganpour reported a reduction in DEA formation and a reduction in metabolic efficiency of amiodaone in hyperlipidemic rats compared to normolipidemic rats [141, 156]. Hereby, our dietary induced obese models demonstrated an increase in HL biomarkers such as cholesterol and triglycerides. Inflammation also can be one of the reasons between the obesity-associated changes in drug metabolizing enzymes and expression. Several studies reported that the increase in plasma inflammatory cytokines can cause a change in expression of some DME due to cytokines effect on the nuclear receptors (such as PXR and CAR) that regulate their expression [109]. Although in our obese models there were no significant changes in proinflammatory mediators levels such as TNFα and adiponectin, leptin increased significantly in the plasma of all groups fed on high caloric diet compared to control [143]. Leptin is known to play an important role in immune response through stimulation of proinflammatory cytokines production. In addition, we might need to measure the concentration of inflammatory cytokines since we could not find a change in the plasma.

Regarding our finding of AM metabolism in intestine, we have reported that DEA formation in intestine was less than in the liver. This is due to the known liver larger capacity and its amounts of the drug metabolizing enzymes compared to intestine. In addition, it has been found that CYP3A intestinal microsomes concentration in human was much higher than in intestinal microsomes of rats and the intestinal CYP3A activities towards CYP3A substrates were different by 2–5 folds between humans and rats [157]. We also found that there are no significant differences in the AM intestinal intrinsic clearance between groups. There is lack of knowledge on the impact of

obesity on the intestinal metabolism of drugs in the literature to compare it with our results.

4.3. The effect of diet induced obesity on the metabolism of bisphenol A in liver and intestine:

Bisphenol A is known to be mainly metabolized by phase II enzymes through UGTs. Our finding demonstrated that the intrinsic clearance of BPA was increased in the liver of all groups fed on high caloric diet compared to control. According to the literature, there is a large discrepancy about the effect of obesity on the expression of UGTs. Several studies have found that the clearance of some drugs that are mainly metabolized by glucuronidation was increased which is consistent with our results of increased clearance of BPA on all groups fed on high caloric diet [64]. A recent study on the pharmacokinetics and pharmacodynamics of propofol in morbidly obese patients undergoing laparoscopic gastric bypass surgery was reported. Propofol is an anesthetic agent mainly metabolized by glucuronidation. It was found that obesity resulted in a significant increase in the clearance of propofol and as a result, its EC50 values (the half of the maximal effect concentration) were decreased significantly compared to lean controls who a similar surgery [158].

Osabe et al. found an increased expression of CAR nuclear receptor with a consequent increased hepatic expression of UGTs in mice fed on high fat and high fructose diet [159]. Moreover, we found a significant decrease in Km values that is indicated a higher affinity of the enzymes for the drug in all obese groups. On the other hand, some studies have proven that obesity due to diet has decreased the hepatic expression

of glucuronidation enzymes in diet induced obesity animal models [67]. It should be noted that in this paper [67], the authors mostly only looked at the mRNA expressions, not the more important protein expression of the drug metabolizing enzymes.

Regarding the glucuronidation process of metabolism in intestine, our results matched the liver findings of increased clearance of BPA in HFD and HFCS fed groups and this may be related to increased expression of UGTs enzymes in the intestine. BPA is known to be mainly metabolized by UGT2B1 in rat liver microsomes. Miyauchi et al. evaluated the protein expression levels of UGTs in human jejunal tissues excised from morbidly obese patients during gastric bypass surgery. They found that UGT1A1, UGT2B15, UGT2B17 exhibited high expression level in these patients [160].

4.4. The effect of genetic obesity on metabolism of amiodarone:

Our results show that obesity due to genetic reasons could significantly influence the metabolism of AM to DEA. We have found that the liver of JCR obese rats had significant increase in the intrinsic clearance of AM compared to lean JCR rats. We assumed that the mechanism of this increase is due to the increased affinity of CYPs enzymes to the drug as we have found that the K_m value was significantly lower in JCR obese rats compared to their lean.

Although a previous study found that genetically obese rats have been shown to be deficient in in vitro hepatic CYPs enzyme activity when they used hexobarbital as a test substrate compared to lean control [161]. Our finding is consistent with other study where the they found that the mRNA and protein levels of some P450 enzymes such as cyp2b10, cyp4a10, and cyp2c29 and CAR nuclear receptor in the liver of

genetically obese/diabetic mice that had a mutation in the leptin receptor gene were increased compared to the control mice [162].

Dietary induced obese models and genetic obese models both had different effects on the metabolism of amiodarone. While dietary induced obese models caused a decrease in the intrinsic clearance of amiodarone, the genetic induced obese models resulted in an increase in the intrinsic clearance. This might be attributed to different factors. Both models might behave differently as each model fed on different diet and there might be different levels of adiposity with each model. Consequently, different effects on metabolism might be obtained using different models of obesity.

The JCR tissues were obtained from Dr. Proctor's laboratory from another study in which both ob/ob and wild type (lean cp/cp) JCR rats were afforded a 30% fat w/w lipid-balanced diet rather for 14 weeks than the rat chow we used in our dietary induced study involving Sprague-Dawley rats. At the time of writing this thesis, we did not have access to the weights of the JCR rats, or serum biochemistry. However, in comparing the AM CLint of the JCR controls (Table 4; 0.97±0.11 μL/min/mg protein) to those of our chow-fed animals (Table 2; 2.65±0.67 μL/min/mg protein), it is clear that the 30% w/w lipid balanced diet rendered them more like our high calorie fed animals than our lean controls fed normal rat chow and water (Table 2). The same is true of the BPA consumption data (Tables 5 and 7). Hence we cannot conclude anything about the effects of cp/cp and ob/ob JCR rats beyond that when they are fed a high fat diet, the liver metabolism appears to be the same.

4.5. The confirmation of BPA glucuronidation by β- glucuronidase hydrolysis reaction:

Since we are measuring the consumption of BPA by measuring the concentration at 0 time and 10 min, we wanted to prove that BPA is already metabolized by glucuronidation to make a conclusion about the effect of obesity on UGTs enzymes. We found that after 6 hr of incubating the hydrolysis enzyme with the microsomes that BPA concentration is almost close to the BPA at 0 time. This confirms that the BPA is metabolized by phase II metabolizing enzymes (UGTs). BPA has been reported to be predominantly metabolized to glucuronide in mammals including rats, monkeys and humans[163-165]. In addition, Yokota et al. reported that BPA is mainly metabolized by UGT2B2 in rat liver microsomes [166].

However, we did not reach the total concentration of BPA at o time after 6 hours of adding the hydrolyzing enzyme. We suggested that the UGTs enzymes are still forming the BPA glucuronide and we might need to block the UGTs activity to stop metabolizing the BPA. In addition, there might be minor pathways of BPA metabolism. Such as BPA sulfation [138]. Moreover, Atkinson et al. and Yoshihara et al. suggested that BPA can be metabolized to 3- hydroxy BPA and BPA o- quinone by CYP450s in rats in vitro and the formation of DNA adducts is significantly reduced by CYPs inhibitors [167-169].

4.6. The effect of genetic obesity on metabolism of bisphenol A:

We could not find any significant changes in the pharmacokinetic parameters V_{max}, K_m, and Cl_{int} for BPA metabolism between lean and obese JCR rats. As pointed out

above, there was a limitation in this assessment because both groups of animals were fed a high fat diet. Nevertheless, the result was consistent with Litterst findings as he reported that UGT enzyme activity was not different between adult male obese and lean Zucker rats [161]. Chaudhary *et al*. studied the effect of genetic obesity on the hepatic conjugation pathways by administering acetaminophen to obese Zucker rats and they reported that the obese Zucker rats demonstrated a higher glucuronidation capacity that was indicated by a higher formation of acetaminophen glucuronide and greater UDP-glucuronosyltransferase activity toward acetaminophen compared with lean controls [170]. Whereas Kim *et al.* found that the expression levels of uridine diphosphate-glucuronosyltransferases 1A1, 1A6, and 2B1 mRNA in liver were significantly lower in obese Zucker rats compared to Sprague-Dawley rats. Consistent with this observation, they found that the intrinsic clearance of two drugs they used as substrates for conjugation pathway in liver microsomes was twofold lower in obese Zucker rats they used as a genetic model to study obesity [75].

Overall, the effects of obesity on drug metabolizing enzymes expression and activity can be inconsistent according to different factors. One of these factors, the species differences as the expression of metabolizing enzymes can be different from mice to rats to human. In addition, genetic induced obesity model versus diet models of obesity as well as the duration that animal models being kept on high caloric diet could result in different stages of obesity and thereby different expression and activity of CYPs and UGTs enzymes. Moreover, the differences in degree and stages of obesity are associated with different pathological and physiological conditions. This

factor is important to consider during investigation of obesity on enzymes activity. Furthermore, diseases associated with obesity can affect genes expression. For example, diabetes has been reported to increase the expression of CYP3A enzymes in mice models having type 1 and type 2 diabetes [162]. Inflammation and alteration in the levels of cytokines have been linked to the change in expression of metabolizing enzymes as Mimura et al. reported that CYP3A4 was down regulated in inflammatory conditions accompanied by high levels of pro-inflammatory cytokines [163]. Also, infection has found to affect the hepatic expression of some enzymes and eventually decrease or suppress their metabolizing activity [164].

Conclusions:

1- Diet induced obesity is associated with a decrease in the functional activity of hepatic CYP450 enzymes.

2- Diet induced obesity is associated with an increase in the functional activity of hepatic and intestinal UGTs enzymes.

3- Obesity due to genetic changes can result in an increase in the functional activity of hepatic CYP450 enzymes.

Future work:

1- To determine the expression of UGTs isoforms in liver and intestinal tissues of obese rats as the assessment of the expression levels of these enzymes could be of great significance for metabolism of many drugs.

2- To determine the expression of nuclear receptors such as CAR and PXR in the liver and intestine of obese rats. These nuclear receptors have an essential role in regulation of phase I and II drug metabolizing enzymes expression.

3- To assess the drug metabolizing activity of JCR genetically-prone obesity using a normal rat chow diet.

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