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Evaluation of Food Effects on the Oral Pharmacokinetics of Rosuvastatin

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A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Physiology and Pharmacology

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Abstract

Rosuvastatin is commonly prescribed for the treatment of hypercholesterolemia and exerts its effect through targeted accumulation in the liver. Current United States and Canadian dosing guidelines indicate no preference for fed or fasted rosuvastatin administration. In this study, we demonstrate for the first time that concomitant administration with food substantially reduced mean plasma rosuvastatin exposure in healthy Canadian East Asian and Caucasian subjects. In mice lower plasma level was also noted with food 2 hours after an oral rosuvastatin dose, while liver concentration was unaffected. Moreover, through retrospective analysis of rosuvastatin patient data, we conclude that taking an oral dose with food as opposed to on an empty stomach, does not significantly affect the cholesterol-lowering capacity of rosuvastatin. Since a common adverse event noted with statin therapy is muscle pain/damage associated with high circulating statin levels, our findings have the potential to serve as a novel and simple strategy for mitigating statin myopathy risk.

Keywords: rosuvastatin, HMG-CoA reductase inhibitors, oral pharmacokinetics, food effect, hepatic uptake transport, drug transporter pharmacogenetics.

Acknowledgments

If I have seen further, it is by standing upon the shoulders of giants. ~ *Sir Isaac Newton*

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Abbreviations

ABC	ATP-binding cassette
AE	adverse effect
AUC	area under the curve
AUC _{0-∞}	AUC extrapolated out to infinity
AUC ₀₋₁₀	AUC from 0 to 10 hours
AUC ₀₋₂	AUC from 0 to 2 hours
BCRP	breast cancer resistance protein
BMI	body mass index
CCIT	Centre for Clinical Investigations and Therapeutics
C _{max}	maximum concentration
CYP	cytochrome P450
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
FDA	Food and Drug Administration
HDL-C	high-density lipoprotein cholesterol
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
LC-MS/MS	liquid chromatography tandem mass spectrometry
LDL-C	low-density lipoprotein cholesterol
LDL-R	low-density lipoprotein receptor
LHSC	London Health Sciences Centre
mRNA	messenger ribonucleic acid

MRP	multidrug resistance-associated protein
NTCP	sodium-dependent taurocholate co-transporting polypeptide
OATP	organic anion-transporting polypeptide
P-gp	P-glycoprotein
PGx	pharmacogenetics
PK	pharmacokinetics
rcf	relative centrifugal force
SD	standard deviation
SLC	solute carrier
SNP	single nucleotide polymorphism
SREBP	sterol regulatory element binding protein
$T_{1/2}$	half-life
TC	total cholesterol
T_{max}	time of maximum concentration

1. INTRODUCTION

1.1. Cardiovascular Disease & Dyslipidemia

Cardiovascular disease, including both heart disease and stroke, is the second leading cause of mortality in Canada (Maclagan *et al.*, 2014). Moreover, cardiovascular disease is associated with disability, decreased quality of life, and an annual economic burden of approximately \$22 billion in lost productivity and direct/indirect healthcare costs (Genest *et al.*, 2009). A major implication in the development of cardiovascular disease is dyslipidemia, or abnormal amount of lipids and lipoproteins in the blood. Specifically, high plasma low-density lipoprotein cholesterol (LDL-C; “bad cholesterol”), low levels of high-density lipoprotein cholesterol (HDL-C; “good cholesterol”), and elevated triglycerides have been indicated as independent predictors of cardiovascular disease (Sharrett *et al.*, 2001; Gordon *et al.*, 1989; Sarwar *et al.*, 2007). Current therapy emphasizes lifestyle modifications (i.e. healthy diet, regular exercise, avoidance of cigarette smoke etc.) and the use of pharmacotherapy to lower circulating LDL-C concentrations (Stone *et al.*, 2014).

Elevated plasma LDL-C is the result of a disturbance in the intricate balance between endogenous and exogenous cholesterol metabolism (Shepard, 2001). Exogenous cholesterol is largely derived from the intestinal absorption of both bile acids and dietary sources; this newly absorbed cholesterol is then packaged with an array of specialty proteins and delivered to the liver for processing (Grundy, 1978). The liver also synthesizes the majority of systemic cholesterol through an endogenous pathway. Cholesterol synthesis begins with the conversion of acetyl CoA to 3-hydroxy-3-methylglutaryl CoA (HMG-CoA), which is then reduced to mevalonic acid. The mevalonic acid is further converted into cholesterol through a complex sequence of condensation reactions (Grundy, 1978). The conversion of HMG-CoA to mevalonic acid by HMG-CoA reductase is widely accepted as the rate-limiting step in cholesterol biosynthesis and, as such, is the target for leading cholesterol-lowering therapies.

Several classes of lipid-modifying medications are currently available, including fibrates (e.g. gemfibrozil, fenofibrate), nicotinic acid (e.g. niacin), bile acid sequestrants (e.g. cholestyramine, colestipol), and cholesterol absorption inhibitors (e.g. ezetimibe). However, statins (i.e. rosuvastatin, atorvastatin, simvastatin etc.) are the most commonly prescribed class of medications for the treatment of hypercholesterolemia (Law *et al.*, 2003).

1.2. Statin Pharmacology

Statin medications are first-line therapy for cardiovascular disease prevention, and an estimated 25.2 million Americans are currently receiving statin therapy (Pencina *et al.*, 2014). However, statin use has been associated with reductions in LDL-C, cardiovascular events, and all-cause mortality, providing strong evidence to support statin use as primary prevention in people at low risk of cardiovascular disease (Taylor *et al.*, 2013). Thus, new guidelines from the American College of Cardiology and the American Heart Association recommend broadening the use of statin therapy to include an additional 30.8 million Americans in addition to those already receiving statins (Stone *et al.*, 2014; Pencina *et al.*, 2014). An overview of statin mechanism of action, their potential for adverse effects, pharmacokinetics (PK), and pharmacogenetics (PGx) is presented below.

1.2.1. Mechanism of Action

Statins exert their pharmacological effect by competitively inhibiting HMG-CoA reductase, decreasing cholesterol production. A lower regulatory pool of cholesterol is sensed by specialized proteins within the endoplasmic reticulum and results in the activation of sterol regulatory element binding protein 2 (SREBP2). In its activated state, SREBP2 acts as a nuclear transcription factor which functions to increase the expression of LDL receptors (LDL-R) on the basolateral membrane of hepatocytes. SREBP2 activation also increases the expression HMG-CoA reductase, however cholesterol production does not increase due to competitive inhibition by the statin. Newly synthesized LDL-R function to clear circulating LDL-C, resulting in lower blood levels of this atherogenic particle (Goldstein & Brown, 2009). A schematic of the primary

mechanism of action of statins is presented in **Figure 1**. In addition to lowering LDL-C, a number of pleiotropic effects of statins have been identified including: increased stability of arterial plaque, decreased oxidative stress and inflammation, and improved endothelial function (Takemoto & Liao, 2001).

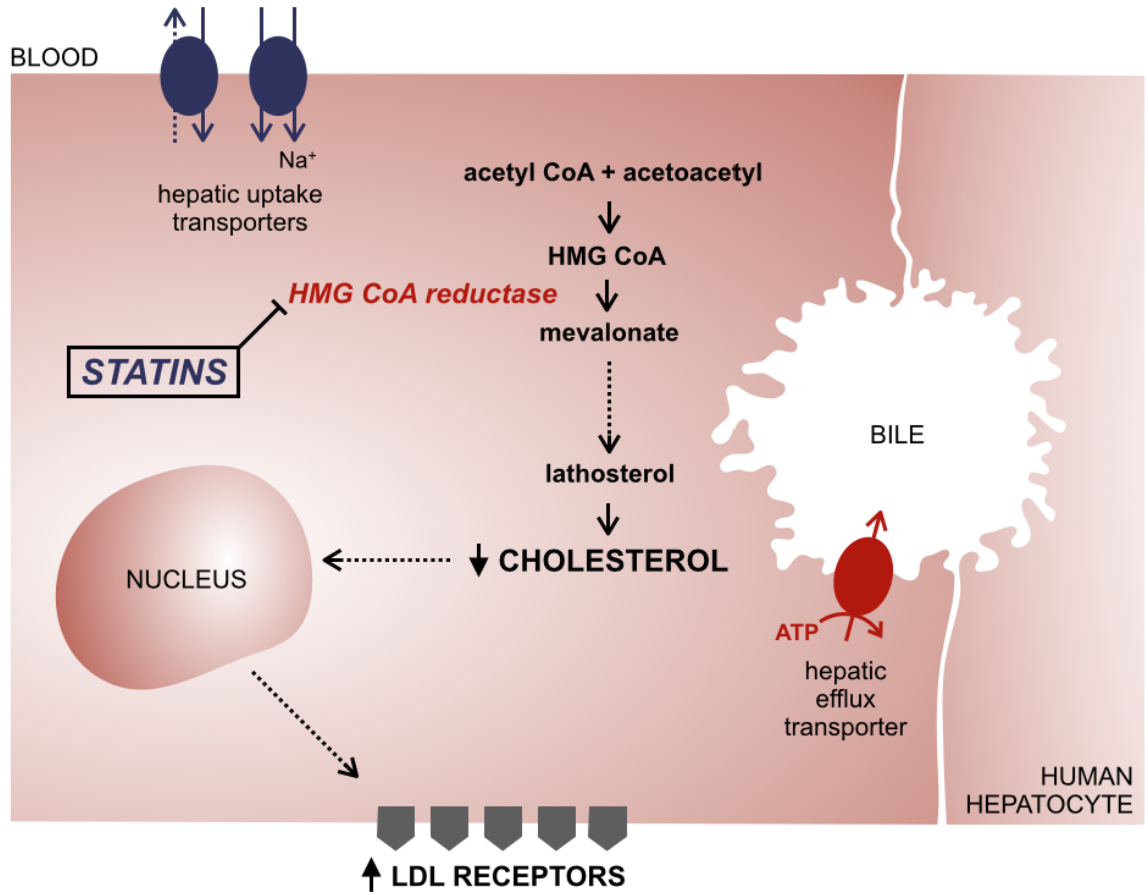


Figure 1. Endogenous cholesterol synthesis and the primary mechanism of action of statin medications. Adapted from DeGorter, 2012a.

In Canada, current guidelines list statins as the first-line pharmacotherapy for the treatment of dyslipidemia. Moreover, guidelines recommend a target LDL-C less than 2 mmol/L, or greater than 50% reduction of LDL-C from untreated baseline (Anderson *et al.*, 2013). This therapeutic target is often achieved through statin monotherapy, as statins have been shown to lower systemic cholesterol by an average of 1.8 mmol/L; this reduction correlates clinically with an average decrease in ischemic heart disease of ~60% and stroke by ~17% (Law *et al.*, 2003). Although an individual's LDL-C reduction is typically used as a measure of statin response, plasma concentrations of lathosterol, a cholesterol biosynthesis intermediate, can be used to indicate the rate of endogenous cholesterol synthesis and, thus, the response to statin therapy (De Cuyper *et al.*, 1993).

1.2.2. Adverse Side Effects

In 2012 an estimated 200 million individuals benefitted from statin use worldwide (Sirtori *et al.*, 2012). The expansive use of statins to lower serum cholesterol is often attributed to their ability to favourably alter multiple aspects of a patient's cholesterol profile and maintain a relatively low adverse-effect (AE) frequency (Evans & Rees, 2002). However, up to 10% of statin patients experience muscle-related AEs and, consequently, require dose adjustment, a switch to an alternate statin medication, or cessation of statin therapy (Joy & Hegele, 2009). Muscle-related toxicities (myopathies) include myalgia (muscle pain) and, occurring in a small number of individuals, rhabdomyolysis (muscle breakdown). Rhabdomyolysis often presents as an asymptomatic elevation of serum muscle enzymes, however in more severe cases resultant acute renal failure and electrolyte abnormalities can be life threatening (Polderman, 2004). Of note, cerivastatin was withdrawn from the market in 2001 due to an increased risk (approximately 12-fold) of both asymptomatic and fatal rhabdomyolysis when compared to other statins (Kashani *et al.*, 2006). It is suggested that the risk of myopathy may be higher for lipophilic statins likely due, in part, to an increased ability to enter muscle cells (Kobayashi *et al.*, 2008). However, even hydrophilic statins such as rosuvastatin are known to cause both myalgia and rhabdomyolysis (García-Rodríguez *et al.*, 2008). Interestingly, the uptake transporter OATP2B1 is present on the sarcolemmal

membrane of human skeletal muscle fibers and is thought to play a key role in modulating skeletal muscle statin exposure and toxicity (Knauer *et al.*, 2010). Substrates for OATP2B1 include atorvastatin, fluvastatin, pravastatin, and rosuvastatin (Knauer, 2012).

Time to onset of statin-induced muscle toxicity varies widely, but on average myopathy is thought to occur around 6 months after beginning therapy (Hansen *et al.*, 2005). Statin-induced myopathy can result in structural damage to muscle fibres, which is thought to persist even if statin use is discontinued (Mohaupt *et al.*, 2009). Although the exact mechanism of statin-induced muscle-related toxicities is unknown (Tomaszewski *et al.*, 2011), myopathy is associated with higher statin dose and increased plasma statin exposure (Jacobson, 2006). Elevated plasma exposure can result from either increased absorption or, more commonly, decreased clearance of the statin. Factors that may affect statin exposure are discussed at length in later sections.

1.2.3. Pharmacokinetics

Statins are commonly administered once daily via an oral dose. Most statins are administered in their active, hydroxy-acid form, however simvastatin and lovastatin are administered as lactone pro-drugs (Schachter, 2005). Although statin medications share a common mechanism of action, they differ in terms of their chemical structure, binding efficacy, pharmacokinetic properties, and lipid-modifying capacity. Pharmacokinetic properties for the six statin medications currently available in Canada are summarized in **Table 1**.

Lipophilicity of statins influences their absorption, distribution, metabolism, and excretion. Atorvastatin, simvastatin, fluvastatin, and lovastatin are relatively lipophilic compounds and can thus readily diffuse through biological membranes. Hydrophilic rosuvastatin and pravastatin, however, require carrier-mediated transport to transverse cellular membranes (Hamelin & Turgeon, 1998). Due to the high expression of statin transporters on hepatocytes, rosuvastatin and pravastatin show greater hepatoselectivity and reduced potential for uptake by peripheral cells when compared to lipophilic statins

Table 1. Pharmacokinetic properties of the six statin medications currently available in Canada.

	Atorvastatin (Lipitor®)	Rosuvastatin (Crestor®)	Simvastatin (Zocor®)	Fluvastatin (Lescol®)	Pravastatin (Pravachol®)	Lovastatin (Mevacor®)
Solubility	Lipophilic	Hydrophilic	Lipophilic	Lipophilic	Hydrophilic	Lipophilic
Oral bioavailability (%)	~14	~20	<5	24-30	~18	~5
Protein binding (%)	>98	~88	95-98	>98	43-54	>95
Time to C _{max} (h)	~1.5	3-5	1.3-2.4	0.4-1.5	0.8-1.5	~2.4
Metabolism	Extensive (predominantly CYP3A4)	Limited	Extensive (predominantly CYP3A4)	Extensive (predominantly CYP2C9)	Limited	Extensive (predominantly CYP3A4)
Half-life (h)	7-14	~19	~2	0.5-2.4	1.3-2.5	~3
Renal excretion (%)	<5	~10	~13	<6	6-20	~10
References	Lennernäs, 2003; Rosenson, 2003	Crouse III, 2008	Mauro, 1993; Rosenson, 2003	Scripture & Pieper, 2001; Rosenson, 2003	Hatanaka, 2000; Rosenson, 2003	Sun <i>et al.</i> , 2002; Rosenson, 2003

(Nezasa *et al.*, 2003). Nevertheless, all statins currently available in Canada demonstrate carrier mediated transport to some extent and therefore expression and activity of these transporters on various tissues greatly impact statin disposition (Rodrigues, 2010).

Statin oral bioavailability ranges from less than 5% for simvastatin up to approximately 30% for fluvastatin (Mauro, 1993; Scripture & Pieper, 2001; Rosenson, 2003). These low values are attributed to extensive gut metabolism and efficient extraction by the liver (Shitara & Sugiyama, 2006). Since statins act in the liver, first-pass hepatic uptake of statin medications is likely more important than oral bioavailability. Uptake transporters expressed on the basolateral membrane of hepatocytes are important for portal extraction of statins by the liver. Specifically, members of the organic anion-transporting polypeptide family (OATP1B1, OATP1B3, OATP2B1), and the sodium-taurocholate cotransporting polypeptide (NTCP) are implicated in statin transport into hepatocytes (DeGorter, 2012a). Following hepatic metabolism, statins are predominantly removed from the body via biliary excretion (Schachter, 2005). ATP-binding cassette (ABC) efflux transporters located on the canalicular membrane of hepatocytes mediate the hepatobiliary excretion of statins (DeGorter, 2012a). **Figure 2** details the hepatic uptake and efflux transporters important for statin disposition.

Both lipophilic and hydrophilic statins exhibit extensive plasma protein binding, ranging from approximately 50% for pravastatin up to greater than 98% for atorvastatin and fluvastatin (Hatanaka, 2000; Lennernäs, 2003; Rosenson, 2003). However, lipophilic statins are more prone to oxidative metabolism by the cytochrome P450 (CYP) family of enzymes (Schachter, 2005). Indeed, atorvastatin, simvastatin, and lovastatin are metabolized by the CYP3A4 isozyme whereas fluvastatin is predominantly metabolized by the CYP2C9 isozyme. Alternatively, hydrophilic pravastatin and rosuvastatin are not significantly metabolized by CYP enzymes (Rosenson, 2003).

Clinical factors including progressive liver and renal disease can alter statin pharmacokinetics. Of note, the package insert for atorvastatin (Lipitor®) suggests that the maximum plasma concentration (C_{max}) and area under the plasma concentration-time

curve (AUC) values are 4-fold greater in patients with well-compensated cirrhosis (Childs-Pugh A disease), and approximately 16-fold and 11-fold higher, respectively, in patients with significant functionally compromised cirrhosis (Childs-Pugh B disease). Product monographs for all six statin medications currently available in Canada state that statin use is contraindicated in individuals with active liver disease. Moreover, dosage guidelines for all available statins, except for atorvastatin, recommend lower doses for individuals with severe (or in some cases moderate) renal impairment. In addition to concomitant disease, other factors that may influence statin pharmacokinetics include genetics, ethnicity, age, sex, and food intake (DeGorter *et al.*, 2013; Gazzero *et al.*, 2012).

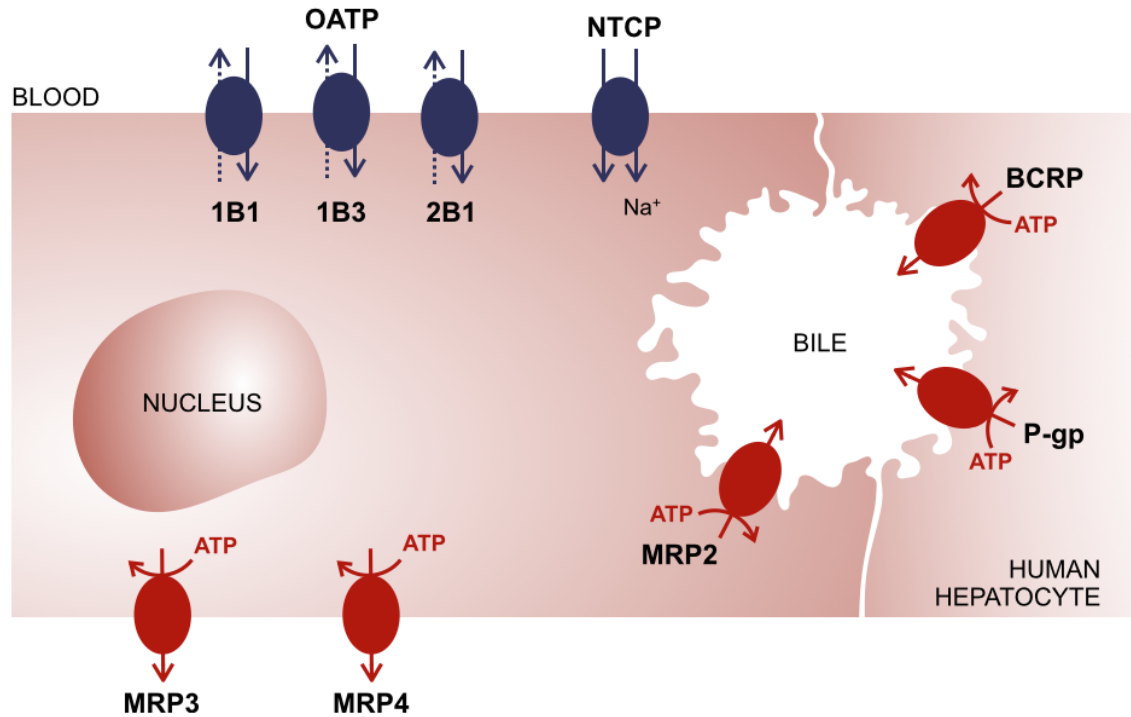


Figure 2. Hepatic uptake and efflux transporters important for statin disposition.

Abbreviations: OATP, organic anion-transporting polypeptide; NTCP, sodium taurocholate cotransporting polypeptide; BCRP, breast cancer resistance protein; P-gp, P-glycoprotein; MRP, multidrug resistance-associated protein. Adapted from DeGorter, 2012a.

1.2.4. Pharmacogenetics

Single nucleotide polymorphisms (SNPs) in statin transporters have been associated with altered disposition of, and response to, statin medications. In particular, loss-of-function SNPs within *ABCG2*, particularly c.421C>A (rs22331142) and c.34G>A (rs2231137), have been found to impact statin pharmacokinetics. *ABCG2* codes for BCRP, an efflux transporter that appears to limit the absorption, entry, or retention of endogenous and exogenous substrates into various tissue compartments (Mao & Unadkat, 2015). *In vitro* work suggests that *ABCG2* c.421C>A significantly impairs BCRP protein expression without altering mRNA expression (Kondo *et al.*, 2004). Clinically, increased AUCs for atorvastatin, fluvastatin, simvastatin lactone, and rosuvastatin were greater in c.421A/A individuals than in c.421C/C individuals (Keskitalo *et al.*, 2009a; Keskitalo *et al.*, 2009b). Moreover, in individuals taking rosuvastatin to manage their hypercholesterolemia, the c.421C>A variant has been found to correlate in a gene dose-dependent manner with reductions in LDL-C (Bailey *et al.*, 2010; Tomlinson *et al.*, 2010). *ABCG2* c.34G>A has been associated with decreased localization of BCRP to the apical membrane of polarized kidney cells *in vitro* (Mizuarai *et al.*, 2004). A recent study in healthy Chinese volunteers shows that mean rosuvastatin AUC and maximum concentration are higher in *ABCG2* c.34AA, c.421AA, and c.34GA/421CA individuals when compared to non-carriers (Wan *et al.*, 2015).

Within *SLCO1B1*, both a loss-of-function polymorphism (c.521T>C; rs4149056) and a gain-of-function polymorphism (c.388A>G; rs2306283) have been shown to impact statin pharmacokinetics. *SLCO1B1* codes for OATP1B1, which is largely expressed at the basolateral membrane of hepatocytes and mediates the uptake of its substrates from portal circulation into the liver (Gong & Kim, 2013). *In vitro* work suggests that c.521T>C reduces transporter expression at the plasma membrane of HeLa cells and consequently impairs transport activity of several OATP1B1 substrates (Tirona *et al.*, 2001). Clinically, c.521T>C has been associated with increased AUC of simvastatin acid in healthy volunteers (Pasanen *et al.*, 2006). A large genome-wide association study revealed c.521T>C as a strong predictor of simvastatin-induced myopathy in statin patients and

estimated an increased 5 year cumulative risk for myopathy in individuals with one or two alleles by 3% and 18% respectively (Link *et al.*, 2008). Moreover, mean atorvastatin and rosuvastatin AUCs were found to be 144% and 65% higher, respectively, in c.521CC individuals when compared to individuals that did not carry the c.521T>C variant (Pasanen *et al.*, 2007). In contrast, *SLCO1B1* c.388A>G has been associated with increased expression of hepatic OATP1B1 in a Caucasian population (Nies *et al.*, 2013). *SLCO1B1* c.388A>G is associated with lower plasma concentrations of various statins (Maeda *et al.*, 2006; Tornio *et al.*, 2015). Moreover, carriers of two variant alleles have shown increased LDL-C reduction in response to atorvastatin treatment when compared with carriers of one or fewer variant alleles (Rodrigues *et al.*, 2011). Furthermore, *SLCO1B1* c.521T>C and c.388A>G are in linkage disequilibrium and can exist as 4 distinct haplotypes (**Table 2**) with varying affects on both statin disposition and response (Gong & Kim, 2013). Of note, *SLCO1B1* c.388A>G + c.521T>C (*15) is associated with decreased transport activity and increased AUC of various statins when compared to wild type (Romaine *et al.*, 2010).

Although select statins are extensively metabolized by members of the CYP enzyme family, evidence regarding the effect of polymorphisms within CYP encoding genes on statin response remains conflictive and inconclusive (Mangravite *et al.*, 2006).

Table 2. Nucleotide and amino acid changes present in the *1a, *1b, *5, and *15 haplotypes of *SLCO1B1*.

Haplotype	Nucleotide change(s)	Amino acid change(s)
*1a	wild type	wild type
*1b	c.388A>G	p.Asn130Asp
*5	c.521T>C	p.Val174Ala
*15	c.388A>G + c.521T>C	p.Asn130Asp + p.Val174Ala

1.3. Statin-Food Effects

Proper adherence to medication regimens can significantly impact health outcomes, and linking drug doses to aspects of an individual's daily routine, such as meal times, can improve compliance (Cramer, 1998). However, taking a medication with food may result in alterations in the physiochemical properties of the drug, and/or changes to the pharmacokinetics or pharmacodynamic profiles of the drug (**Figure 3**). These alterations may have clinical implications such as adverse drug reactions or decreased therapeutic efficacy (Singh, 1999). Moreover, the caloric and nutrient contents of a co-administered meal, along with the physical properties of the meal (size, temperature etc.), can influence a medication's gastrointestinal-transit time, luminal dissolution, tissue permeability, and systemic availability. Common mechanisms for food-drug interactions include changes in gastric emptying or pH, alterations in bile flow and/or splanchnic blood flow, physical or chemical interactions between the medication and food components, and alterations in drug metabolism and/or transport (Winstanley & Orme, 1989; Singh, 1999). The Food and Drug Administration recognized the potential for food to effect the oral pharmacokinetics of new medications and has established standards for the design and execution of clinical food-effect studies (Food and Drug Administration, 2002).

Grapefruit juice-drug interactions are perhaps the most extensively studied. Grapefruit juice can increase systemic drug exposure by up to 14-fold by inhibiting CYP3A-mediated metabolism within the small intestine (Won *et al.*, 2010). The CYP3A-subfamily is thought to be involved in the oxidative metabolism of over 50% of medications, including three of the six statins currently available in Canada (Won *et al.*, 2010). Indeed, product monographs for Lipitor® (atorvastatin), Zocor® (simvastatin), and Mevacor® (lovastatin), caution against grapefruit juice consumption. Aside from grapefruit juice effects, few statin-food interactions have been reported. Food interactions with the six statins available in Canada (lovastatin, pravastatin, fluvastatin, simvastatin, atorvastatin, and rosuvastatin) are reviewed below.

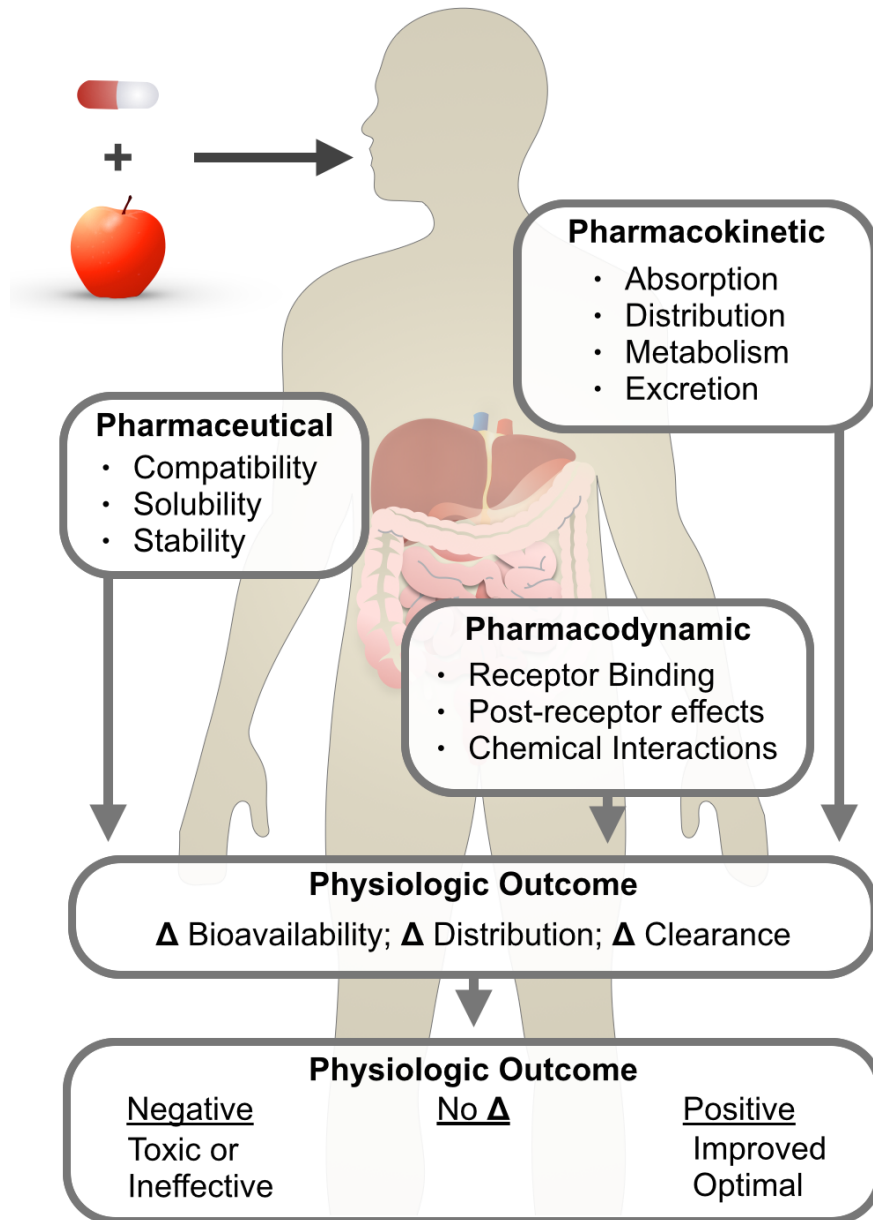


Figure 3. Working model of drug-food interactions. Adapted from Boullata & Hudson, 2012.

1.3.1. Lovastatin

When lovastatin was given under fasted conditions, plasma concentrations of the parent drug and its active metabolite (lovastatin acid) were approximately two-thirds those found when lovastatin was administered with food (Mevacor® product monograph). Thus, current recommendations suggest that lovastatin be taken with food in order to enhance bioavailability of the medication. However, ingestion of a standard breakfast with lovastatin extended release tablets decreased C_{max} and AUC by approximately 40% when compared to values obtained from fasted administration (Sun *et al.*, 2002). High intake of dietary fibre has been shown to reduce the LDL-C lowering effect of lovastatin in hyperlipidemic patients (Richter *et al.*, 1991). Moreover, grapefruit juice can significantly increase plasma concentrations of lovastatin and lovastatin acid, likely due to inhibition of intestinal CYP3A4 (Kantola *et al.*, 1998).

1.3.2. Pravastatin

Administration of pravastatin with food resulted in a 49% and 31% reduction in C_{max} and AUC, respectively, when compared to administration one hour before a meal, without affecting the LDL-C actions of pravastatin (Pan *et al.*, 1993). Consequently, current recommendations suggest that pravastatin may be taken without regard to food. Not surprisingly, due to its limited metabolism, grapefruit juice does not significantly affect pravastatin pharmacokinetics (Lilja *et al.*, 1999).

1.3.3. Fluvastatin

Current dosing guidelines indicate no preference for fed or fasted administration of fluvastatin. Peak serum concentrations of fluvastatin were lower in primary hypercholesterolemic patients that took their dose with an evening meal when compared to a fasted administration. However, taking fluvastatin with or without food did not significantly affect its extent of bioavailability or the ability to lower LDL-C within these patients (Dujovne & Davidson, 1994). Interestingly, one study found that fluvastatin treatment in rats fed a high-fat diet led to increased systemic exposure, skeletal muscle toxicity, and hepatic steatosis accompanied by severe hepatotoxicity (Sugatani *et al.*,

2010). Authors from this work also found that the expression of mRNA and protein of the rat hepatic uptake transporter, *Oatp2*, was suppressed in high-fat fed rats administered fluvastatin. Reduced hepatic uptake could explain the increased systemic exposure and muscle toxicity, whereas hepatic steatosis and hepatotoxicity within rats have been shown to result from prolonged high-fat feeding (Sugatani *et al.*, 2010).

1.3.4. Simvastatin

Although the data are sparse, a few reports indicate that concomitant food administration with simvastatin does not significantly affect simvastatin pharmacokinetics or therapeutic action (Corsini *et al.*, 1999; Zocor® product monograph). Of note, grapefruit juice increased the mean C_{max} and AUC of unchanged simvastatin by approximately 9-fold and 16-fold respectively. Moreover, mean peak serum concentration and total systemic exposure of simvastatin acid were also increased by approximately 7-fold each by grapefruit juice consumption (Lilja *et al.*, 1998). Additionally, rats fed capsaicin (a main ingredient in chili peppers) daily for one week before simvastatin treatment demonstrated reduced C_{max} and AUC for both simvastatin and its acid metabolite when compared to controls. Authors from this work hypothesize that capsaicin may be a potent inducer of select CYP3A enzymes and thus cause increased metabolism of simvastatin (Zhai *et al.*, 2013).

1.3.5. Atorvastatin

Guidelines for atorvastatin dosing indicate no preference for fed or fasted administration. In healthy volunteers, administration of atorvastatin with food resulted in C_{max} and AUC values that were 48% and 13% lower, respectively, than values attained when atorvastatin was administered without food (Radulovic *et al.*, 1995). In another study, administration with food was found to similarly affect atorvastatin pharmacokinetics, but LDL-C reduction was similar when atorvastatin was administered with or without food (Whitfield *et al.*, 2000). Moreover, grapefruit juice significantly increased plasma concentrations of atorvastatin acid and atorvastatin lactone (Lilja *et al.*, 1999).

1.3.6. Rosuvastatin

Current United States and Canadian manufacturer guidelines indicate no preference for fed or fasted rosuvastatin administration. Moreover, within the Food and Drug Administration (FDA) approval package for Crestor® (rosuvastatin calcium), it states that administration with food does not alter the systemic exposure of rosuvastatin (Center for Drug Evaluation and Research, 2012). However, one study performed in healthy Chinese volunteers indicates a drastic decrease in rosuvastatin plasma concentrations (both C_{\max} and AUC) when rosuvastatin is administered with food compared to fasted administration (Li *et al.*, 2009). Similarly, a recent study in dogs demonstrated lower statin plasma exposure when rosuvastatin is administered with food, including both low- and high-fat meals, with the latter having the greater effect on rosuvastatin plasma concentrations (Baek *et al.*, 2013).

2. RATIONALE, SPECIFIC AIMS, & HYPOTHESES

2.1. Rationale

Statin medications target the liver to competitively inhibit the rate-limiting enzyme in the cholesterol biosynthesis pathway, HMG-CoA reductase, and are commonly prescribed to manage hypercholesterolemia. The extensive use of statins is often attributed to their ability to significantly reduce cardiovascular event risk by lowering plasma levels of low-density lipoprotein cholesterol (Law *et al.*, 2003). A notable barrier to statin therapy is skeletal muscle toxicity, which is associated with increased systemic statin exposure (Jacobson, 2006). Indeed, up to 10% of statin patients experience some degree of muscle pain or weakness (Joy & Hegele, 2009).

Rosuvastatin is a synthetic statin that demonstrates high hepatic selectivity and minimal metabolism. Moreover, rosuvastatin is one of the most potent statins and has demonstrated superior cholesterol lowering abilities when compared to other statins on the market (Barakat *et al.*, 2013; McKenney, 2005). Consequently, many physicians have taken to prescribing rosuvastatin ahead of other statin medications, making rosuvastatin the number one most prescribed statin (and the second most prescribed medication overall) in Canada in 2010 (IMS Health Canada, 2010).

Current dosing guidelines for rosuvastatin indicate no preference for fed or fasted administration (Center for Drug Evaluation and Research, 2012), but recent pharmacokinetic data suggest that rosuvastatin systemic exposure is significantly reduced when administered with food (Li *et al.*, 2009; Baek *et al.*, 2013). It was concluded that this food effect was likely a result of decreased intestinal absorption of rosuvastatin (Li *et al.*, 2009; Baek *et al.*, 2013). However, lower systemic exposure could also be explained by increased hepatic clearance of rosuvastatin.

Our group was the first to demonstrate that a liver-specific uptake transporter known as NTCP, was capable of mediating the hepatic uptake of rosuvastatin (Ho *et al.*, 2006). Although the OATP family of transporters, including OATP1B1, has been viewed as the

principal mediators for the hepatic uptake of statins, previous data in our lab demonstrated that NTCP may account for nearly one third of rosuvastatin uptake (Ho *et al.*, 2006). NTCP is a major transporter involved in the enterohepatic recirculation of bile acids, and its expression and activity are highly regulated by the presence or absence of bile acids after food ingestion. We now know that much of its activity is regulated in a highly dynamic fashion through insertion and retrieval from the hepatocyte basolateral membrane, allowing efficient management of a high bile-acid load associated with food ingestion (Anwer & Stieger, 2014). Due to the fact that NTCP is expressed on the basolateral membrane of hepatocytes, increased expression or activity would aid in the clearance of statins from the portal circulation, while lowering circulating statin concentrations. Therefore, although concomitant administration of food may decrease intestinal absorption of rosuvastatin, food may also stimulate rosuvastatin hepatic uptake. Furthermore, since statins function through targeted accumulation in the liver (Ho *et al.*, 2006), a treatment regime which allows for increased hepatic uptake (and consequently, reduced systemic exposure) of the statin may increase efficacy while mitigating statin-induced muscle adverse effects.

To date, food effect for rosuvastatin has been evaluated only in dogs and in a Chinese population (Baek *et al.*, 2013; Li *et al.*, 2009), in which rosuvastatin clearance is known to differ from Caucasians (Lee *et al.*, 2005). Moreover, the study performed in Chinese subjects used generic rosuvastatin provided by DYNE PHARMA (Shandong, China), rather than the brandname product, Crestor®, commonly used in North America. Therefore, the objectives for the present work were to determine the effect of concomitantly administered food on rosuvastatin pharmacokinetics in Canadian East Asian individuals and to elucidate this effect, for the first time, in Caucasian individuals. Moreover, we sought to investigate the effect of concomitant food administration with rosuvastatin dose on the lipid profiles of statin patients. Finally, we wished to examine a possible food effect within mice.

2.2. Specific Aims & Hypotheses

2.2.1. Specific Aim 1

Determine the effect of concomitant food administration, and type of meal administered (high-fat versus low-fat), on plasma rosuvastatin concentrations in healthy Canadian East Asian and Caucasian volunteers. We hypothesize that both C_{\max} and AUC will be higher when rosuvastatin is administered without food when compared to fed administration. As rosuvastatin and bile acids share hepatic uptake transporters (Ho *et al.*, 2006), we expect that fat content of a co-administered meal will affect rosuvastatin pharmacokinetics. Moreover, we hypothesize that bile acids will stimulate hepatic entry of rosuvastatin and, since a larger bile-acid load accompanies the ingestion of a high-fat meal (Marciani *et al.*, 2013), we expect that plasma rosuvastatin AUC will be lowest in the high-fat state. To test this hypothesis, we performed a prospective, crossover pharmacokinetic study in 23 healthy Caucasian and East Asian volunteers (Section 3.1).

2.2.2. Specific Aim 2

Investigate the effect of concomitant food administration with rosuvastatin dose on the lipid profiles of statin patients. We hypothesize that there will be greater liver retention of rosuvastatin in patients who took their dose with food, even among those who appear to have lower systemic statin exposure. We expect that this will be reflected as equal LDL-C lowering when compared to individuals who took their rosuvastatin on an empty stomach. To test this hypothesis, we retrospectively analyzed data from 157 previously recruited individuals from the London Health Sciences Centre (LHSC) Lipid Clinic (Section 3.2).

2.2.3. Specific Aim 3

Determine whether liver-plasma ratios of rosuvastatin are different when an oral dose is administered with food compared to a fasted administration. We hypothesize that the plasma rosuvastatin AUC and C_{\max} will be lower and liver rosuvastatin level will be higher in a fed state. We expect the liver-to-plasma ratio to be higher when

rosuvastatin is administered with food compared to a fasted administration. To test this hypothesis, we performed an *in vivo* pharmacokinetic study in wild-type C57BL/6 mice (Section 3.3).

3. METHODS

3.1. Human Pharmacokinetic Study

3.1.1. Study Design

A prospective, open, randomized, crossover pharmacokinetic study performed in healthy Caucasian and East Asian volunteers was conducted at the Centre for Clinical Investigations and Therapeutics (CCIT), LHSC. All individuals within the East Asian cohort were of Chinese or Korean descent (self reported). All participants provided written informed consent. The study was approved by the Research Ethics Board of the University of Western Ontario, London, Canada (Appendix A). Participants were deemed healthy by the study physician upon physical examination, a brief medical history, and analysis of routine serum chemistry (including a complete cholesterol profile). Moreover, participants were not to be on any prescription or non-prescription medications (with the exception of oral contraceptives) within one month prior to and during the study. Volunteers deemed healthy were invited back to complete three separate study days (approximately 10.5 hours each). Volunteers were asked to fast beginning at 12 am the morning of each study day, refrain from alcohol consumption 24 hours prior to each study day, and refrain from caffeine consumption on each study day. Furthermore, urine pregnancy tests were conducted for female volunteers prior to drug administration on each study day. Rosuvastatin (Crestor®), 10 mg, was administered on three separate study days with one of three standardized breakfasts: fasting, low-fat (20.8% fat, 10.9% protein, 58.1% carbohydrates), or high-fat (46.9% fat, 10.1% protein, 39.91% carbohydrates). Details regarding the standardized breakfasts can be found in **Table 3**.

The order in which each subject underwent the three study days was randomized, and a washout period of one week was required between study days. On each of the three study days, 5 mL blood samples were collected into ethylenediaminetetraacetic acid (EDTA) tubes prior to drug administration and at 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, and 10 hours after drug administration (**Figure 4**). Blood samples were then centrifuged at 4 °C, 2000 rcf for 10 min to separate plasma and cellular components. Plasma was aliquoted into

corresponding cryovials and stored at -80 °C. At the beginning of the first study day, an additional 5 mL blood sample was collected into an EDTA tube for subsequent DNA extraction and genotype analysis; this sample was stored as whole blood at 4 °C. Subjects were provided with a meal of their choosing 5 hours after drug administration; each subject was then provided with the same meal for the remaining study days.

Table 3. Meal components for high-fat and low-fat breakfasts.

High-fat Meal				
	kcal	Fat (g)	Protein (g)	Carb (g)
Carrot muffin	310.7	13.53	5.01	24.05
Becel	70	8	0	0
Apple juice	56	0.13	0.07	13.96
Milk - 3.25%	80	4	4.5	6
Banana	105	0.4	1.3	27
Cheddar cheese	90	11	7	0
Total	711.7	37.06	17.88	71.01
Low-fat Meal				
	kcal	Fat (g)	Protein (g)	Carb (g)
Blueberry muffin	300	12	4	23
Strawberry jam	50	0	0.1	13
Apple juice	56	0.13	0.07	13.96
Milk - 1%	50	1.25	4.5	6
Banana	105	0.4	1.3	27
Vanilla greek yogurt	100	1.5	8	13
Total	661	15.28	17.97	95.96

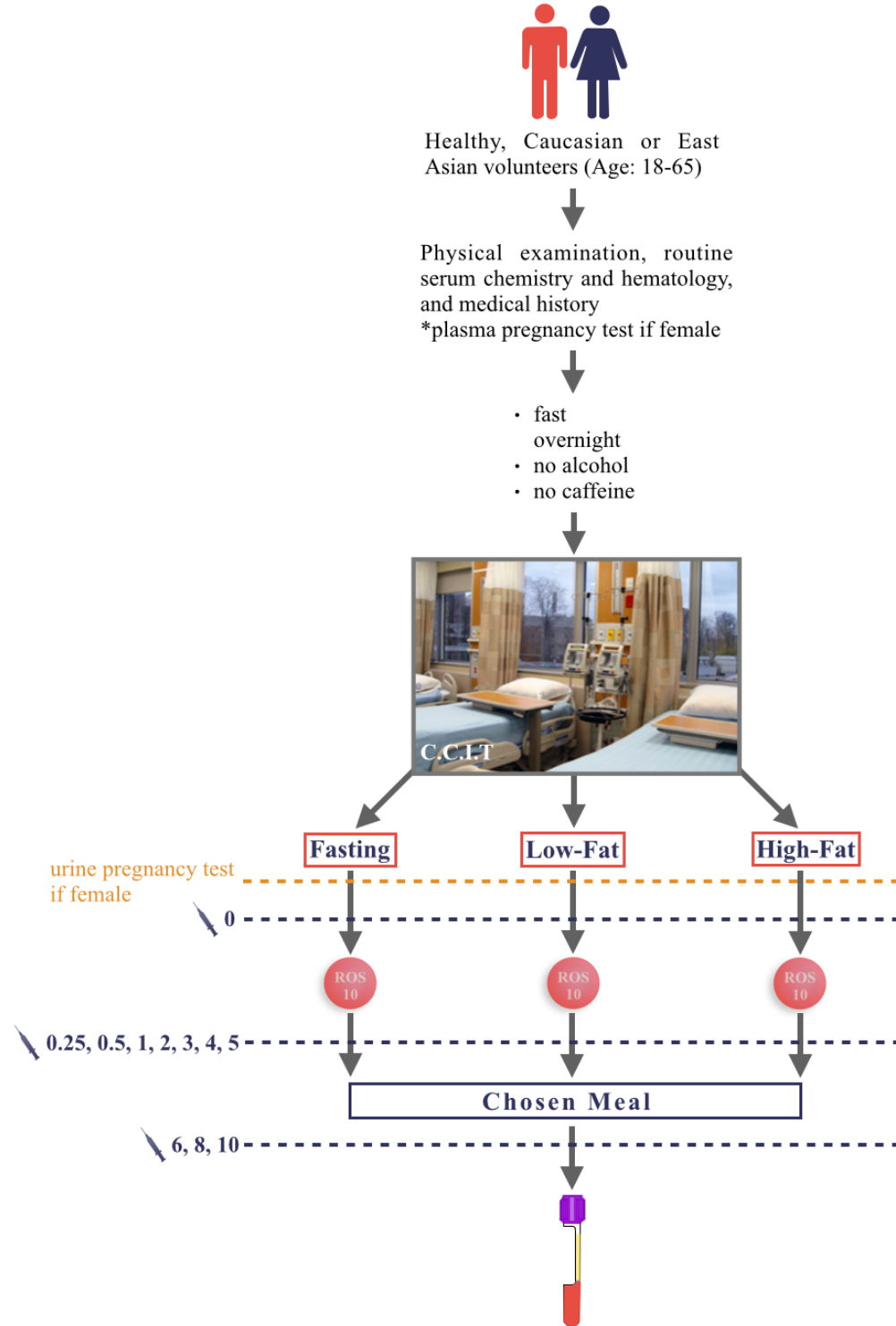


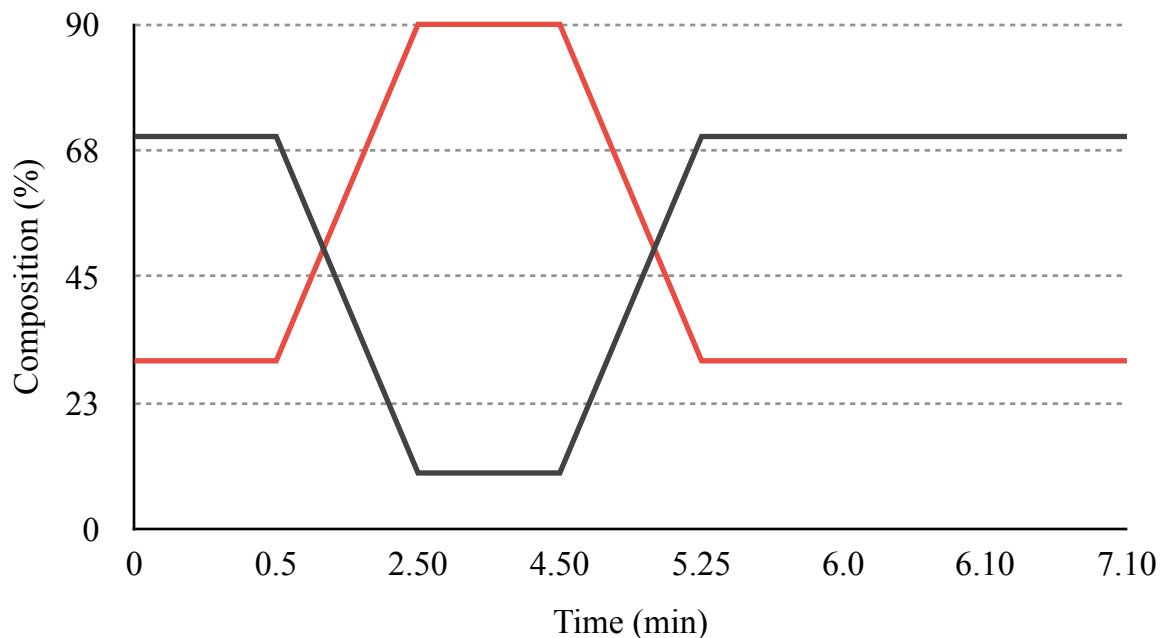
Figure 4. Human pharmacokinetic study design. Abbreviation: CCIT, Centre for Clinical Investigations and Therapeutics.

3.1.2. Determination of Statin Concentrations

Plasma rosuvastatin concentrations were determined using an adapted version of a previously described LC-MS/MS method (DeGorter *et al.*, 2012b). Standard curve values were created using blank human plasma (K2 EDTA, BioreclamationIVT, New York, NY, USA) and rosuvastatin-calcium salt (Toronto Research Chemicals, North York, ON, Canada). Standard aliquots and plasma samples (100 μ L) from each time point were mixed with 300 μ L acetonitrile containing an internal standard (rosuvastatin-d6, Toronto Research Chemicals, North York, ON, Canada) to precipitate any residual proteins. This mixture was then centrifuged for 20 minutes at 14000 rpm at 4°C. The supernatant was removed and diluted 1:1 in 0.05% formic acid in water. A 50 μ L aliquot of each sample was then injected through a Vantage triple-quadrupole mass spectrometer attached to a TLX2 high-performance liquid chromatography system (Thermo TSQ Vantage, Thermo Scientific, Pittsburgh, PA; details in **Table 4** and **Figure 5**). Analytes were separated by reverse-phase chromatography (Kintex 5 μ m EVO C18 100 Å with guard, 50-3.0 mm, Torrance, CA, USA) using gradient elution with 0.05% formic acid in water and acetonitrile starting at a ratio of 70:30 with a gradient to a ratio of 10:90 (Agilent 1200, Agilent, Santa Clara, CA, USA). The flow rate was set at 0.5 mL/min and the total run time for each sample was 6.5 min. The lower limit of quantification for rosuvastatin was 0.1 ng/ml. Assay bias and precision (coefficient of variation) were 1.1% and 8.0%, respectively.

Table 4. Vantage triple-quadrupole mass spectrometer attached to a TLX2 high-performance liquid chromatography system method settings.

Parameter	Setting	
Run duration	6.50 min	
Ionspray voltage (positive ion mode)	+3000 V	
Sheath gas	50 Arb.	
Ion sweep gas	-1 Arb.	
Auxiliary gas	20 Arb.	
Capillary temperature	350°C	
Collision pressure	1 mTorr	
Column	Kintex 5 μ EVO C18 100 Å with guard, 50-3.0 mm	
Rosuvastatin	Q1 mass	482.1
	Q3 mass	258.2
	Collision energy	-34 V
	Ionization mode	Positive
d-Rosuvastatin	Q1 mass	488.0
	Q3 mass	264.3
	Collision energy	-34 V
	Ionization mode	Positive



Time	Flow (mL/min)	A(%)	B(%)
0.00	0.5	70.0	30.0
0.50	0.5	70.0	30.0
2.50	0.5	10.0	90.0
4.50	0.5	10.0	90.0
5.25	0.5	70.0	30.0
6.00	0.5	70.0	30.0
6.10	0.5	70.0	30.0
7.10	0.5	70.0	30.0

A = 0.05% formic acid in H₂O

B = organic acetonitrile

Figure 5. Vantage triple-quadrupole mass spectrometer attached to a TLX2 high-performance liquid chromatography system mobile phase composition and gradient method.

3.1.3. Pharmacokinetics and Statistical Analysis

For each treatment condition, the area under the plasma rosuvastatin concentration-time curve from 0 to 10 hours (AUC_{0-10}), AUC extrapolated out to infinity ($AUC_{0-\infty}$), the maximum plasma concentration (C_{max}), and time of maximum concentration (T_{max}) were calculated using PKSolver (add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel). The data were first tested for normality using the D'Agostino and Pearson omnibus normality test. Non-normal datasets were log-transformed before subsequent comparison by one-way ANOVA and Tukey's multiple comparisons test. Moreover, using the combined dataset, AUC_{0-10} , $AUC_{0-\infty}$, C_{max} , and T_{max} for each treatment condition were compared between sexes. For this comparison, normally distributed data were compared using an unpaired t-test with equal standard deviations, whereas non-parametric data were compared using the Mann-Whitney test. All statistical analyses were performed using GraphPad Prism 6 (GraphPad, La Jolla, CA, USA).

3.1.4. DNA Extraction and Genotype Analysis

DNA was isolated from whole blood samples using the MagNA Pure Compact Nucleic Acid Isolation Kit I and MagNA Pure Compact Instrument (Roche, Indianapolis, IN, USA). DNA samples were analyzed using TaqMan allelic discrimination assays for the following polymorphisms: *SLCO1B1* c.388A>G (*1b), *SLCO1B1* c.521T>C (*5), *ABCG2* c.421C>A, and *ABCG2* c.34G>A. Patient DNA was loaded in duplicate into 96-well plates with Applied Biosystems PCR Master Mix and polymorphism-specific SNP Genotyping Assay Mix and the assay was performed using the Applied Biosystems Viia 7 Real-Time PCR System (Thermo Fisher Scientific, Mississauga, ON, Canada). Analysis of TaqMan results and assignment of volunteer genotypes for each analyzed polymorphism were completed using Viia 7 software (Thermo Fisher Scientific).

$AUC_{0-\infty}$ and C_{max} values were then compared between variant-allele carriers (both heterozygous and homozygous) and wild-type individuals for each SNP. The data were tested for normality using the D'Agostino & Pearson omnibus normality test; normally

distributed data were compared using an unpaired t-test with equal standard deviations, whereas non-parametric data were compared using the Mann-Whitney test.

3.2. Retrospective Analysis of Patient Data

3.2.1. Study Design

The study population included 157 previously recruited individuals from the LHSC Lipid Clinic from August 2009 to May 2011 (DeGorter *et al.*, 2013). All patients provided informed written consent. Each patient was on daily rosuvastatin therapy at the time of enrolment and provided a blood sample as previously described (DeGorter *et al.*, 2013). Data collected upon enrolment included daily dose of rosuvastatin, time of sample collection, and time since last dose. Plasma concentrations of lathosterol and total cholesterol (TC) were measured from the provided blood samples as previously described (DeGorter *et al.*, 2013). Clinically measured LDL-C levels were used where possible; if LDL-C values were not recorded, these values were obtained using the Friedewald equation and clinically recorded HDL-C, TC, and triglyceride values.

Patients who reported taking their last rosuvastatin dose between 5-9 am (inclusive) or 5-7 pm (inclusive) were presumed to have taken the medication with food (n=75). Patients who reported taking their last rosuvastatin dose at or after 8 pm were presumed to have taken the medication without food (n=82). Individuals who reported taking their last rosuvastatin dose at alternate times were not included for analysis. Moreover, we presumed that these patients were likely to routinely take their rosuvastatin dose under similar conditions.

3.2.2. Statistical Analysis

The average dose of rosuvastatin was compared between individuals presumed to have taken their rosuvastatin with food to those presumed to have taken their dose without food. Patients were further categorized based on dose: 5 mg, 10 mg, 20 mg, and 40 mg daily rosuvastatin. For each dose, plasma LDL-C (n=146), lathosterol (n=154), and total cholesterol (n=154) concentrations were compared between individuals presumed to have

taken their last dose with food and those presumed to have taken their last dose without food. The data were tested for normality using the D'Agostino & Pearson omnibus normality test; normally distributed data were compared using an unpaired t-test with equal standard deviations, whereas non-parametric data were compared using the Mann-Whitney test.

3.3. Mouse Pharmacokinetic Study

3.3.1. Study Design

An *in vivo* pharmacokinetic study was performed in two groups (fed and fasted) of wild-type C57BL/6 mice (Jackson Laboratory, Bar Harbour, MA; 10 weeks old; ~26 g; 5 per group). The mice were housed in a temperature-controlled environment with a 12 h light/dark cycle where they received standard murine chow and water *ad libitum*. Six hours prior to drug dosing, all food was removed from the fasting mouse group and the bedding was changed to ensure that there was no residual food in the cages. The bedding was also changed in the cages of the fed mice. All mice were dosed 10 mg/kg rosuvastatin in phosphate buffered saline (total volume of 200 μ L) by oral gavage. Approximately 30 μ L of blood was collected prior to rosuvastatin dose (0 time point), and at 7.5, 15, 30, and 60 min after drug dosing. All blood was collected using a heparinized pipet after saphenous vein puncture. At 120 min after drug dosing, the mice were euthanized by isoflurane and the remaining blood was collected into EDTA-containing tubes via cardiac puncture. All blood samples were centrifuged at 4 °C, 2000 rcf for 10 min to separate plasma and cellular components. Plasma was aliquoted into corresponding cryovials and stored at -80 °C. Livers from all mice were excised postmortem, rinsed in phosphate-buffered saline, blotted, and weighed; liver samples were flash frozen in liquid nitrogen and stored at -80 °C. This study protocol was approved by the Animal Use Subcommittee of the University of Western Ontario, London, Canada (Appendix B). **Figure 6** details this study design.

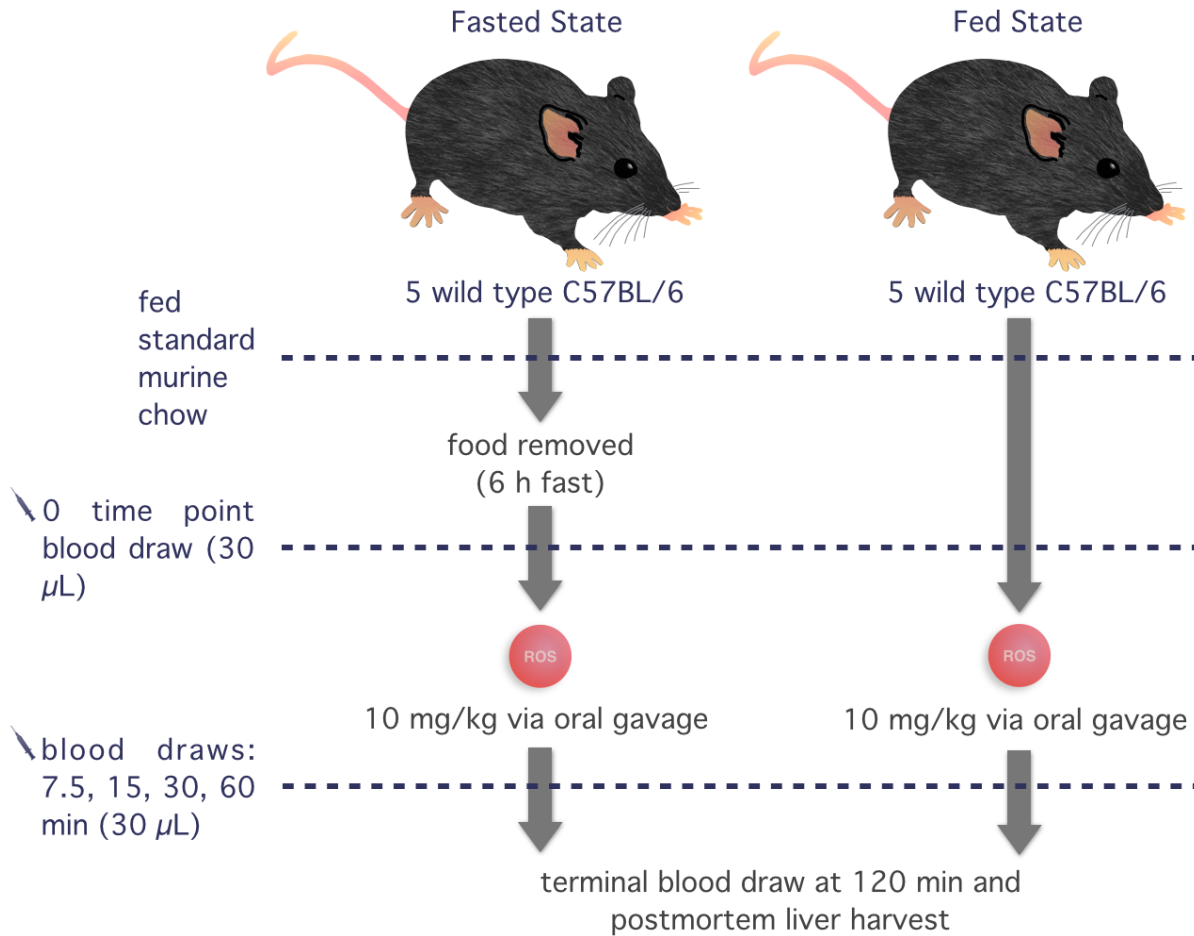


Figure 6. Mouse pharmacokinetic study design.

3.3.2. Determination of Statin Concentrations

Mouse plasma and liver rosuvastatin concentrations were determined using an adapted version of the LC-MS/MS method described in Section 3.1.2. In brief, two sets of standard curve values were prepared. The first set was used when analyzing plasma samples and was prepared using blank mouse plasma (K2 EDTA, BioreclamationIVT, New York, NY, USA) and rosuvastatin-calcium salt. The second set of standard curve values was used when analyzing liver samples and was prepared using homogenized, wild-type, untreated, C57BL/6 liver samples and rosuvastatin calcium salt. Liver samples and standards were homogenized 1:1 (weight to volume) in 0.05% formic acid in water. Plasma samples and liver homogenate samples (5 μ L of each) were precipitated using 20 μ L acetonitrile containing internal standard (rosuvastatin-d6) and centrifuged for 20 min at 14000 rpm at 4°C. The supernatant from each sample was then diluted 1:2 in 0.05% formic acid in water and analysis was carried out as described in Section 2.1.2. Note injection volume was reduced to 30 μ L for analysis.

3.3.3. Pharmacokinetics and Statistical Analysis

AUC from 0 to 2 hours (AUC_{0-2}), AUC extrapolated out to infinity ($AUC_{0-\infty}$), the maximum plasma concentration (C_{max}), time of maximum concentration (T_{max}), and half-life ($T_{1/2}$) were calculated using PKSolver. Pharmacokinetic data from the fed and fasted mouse groups were compared using the Mann-Whitney test. Liver-to-plasma ratios were calculated by dividing the tissue rosuvastatin concentration by the plasma concentration at the final sampling time point (2 hour). Mean plasma rosuvastatin concentrations at 2 hours, liver rosuvastatin concentrations, and liver-to-plasma ratios were compared between fed and fasted mouse groups using the Mann-Whitney test. Statistical analysis was performed using GraphPad Prism 6.

4. RESULTS

4.1. Human Pharmacokinetic Study

4.1.1. Demographic and Recruitment Data

Healthy Canadian East Asian and Caucasian individuals were recruited to complete a pharmacokinetic study investigating the effect of concomitantly administered food on the oral pharmacokinetics of rosuvastatin. Data that we collected from 11 individuals was used to calculate that a sample size of 20 individuals total would provide 80% power to observe a 35% difference in plasma rosuvastatin levels at 95% confidence. In total, 27 healthy participants were enrolled in this study, and 23 participants completed all three study days (**Figure 7**). Of the 14 Caucasian subjects, 6 males and 5 females completed the study, and 3 females were withdrawn before study completion. Thirteen East Asian subjects were enrolled; 6 males and 6 females completed all three study days and 1 female did not complete the study.

Median age (range) and mean body mass index (BMI) \pm SD for the Caucasian cohort were 22 (21-59) years and 25.92 ± 3.13 kg/m² respectively (**Table 5**). These values were slightly lower in the East Asian cohort with a median age of 21 (20-23) years and BMI of 22.90 ± 4.01 kg/m² (**Table 6**). Average total cholesterol, circulating triglycerides, HDL-C, and LDL-C were similar between cohorts and average values for each cohort were within or near the lipid reference ranges as specified by the London Laboratory Services Group (2008). Of note, average LDL-C \pm SD for the Caucasian cohort was 2.58 ± 0.68 mmol/L and the reference range for LDL-C is ≤ 2.5 mmol/L. Moreover, average HDL-C values were slightly above the 1.3-1.55 mmol/L reference for both cohorts: mean HDL-C \pm SD was 1.64 ± 0.45 mmol/L for the Caucasian cohort and 1.59 ± 0.27 mmol/L for the East Asian cohort. Further details regarding subject demographics can be found in **Table 5** and **Table 6**. Note that total cholesterol, triglyceride, HDL-C, and LDL-C values presented in these tables were determined by LHSC Core Laboratories as part of the initial serum screening process for this study.

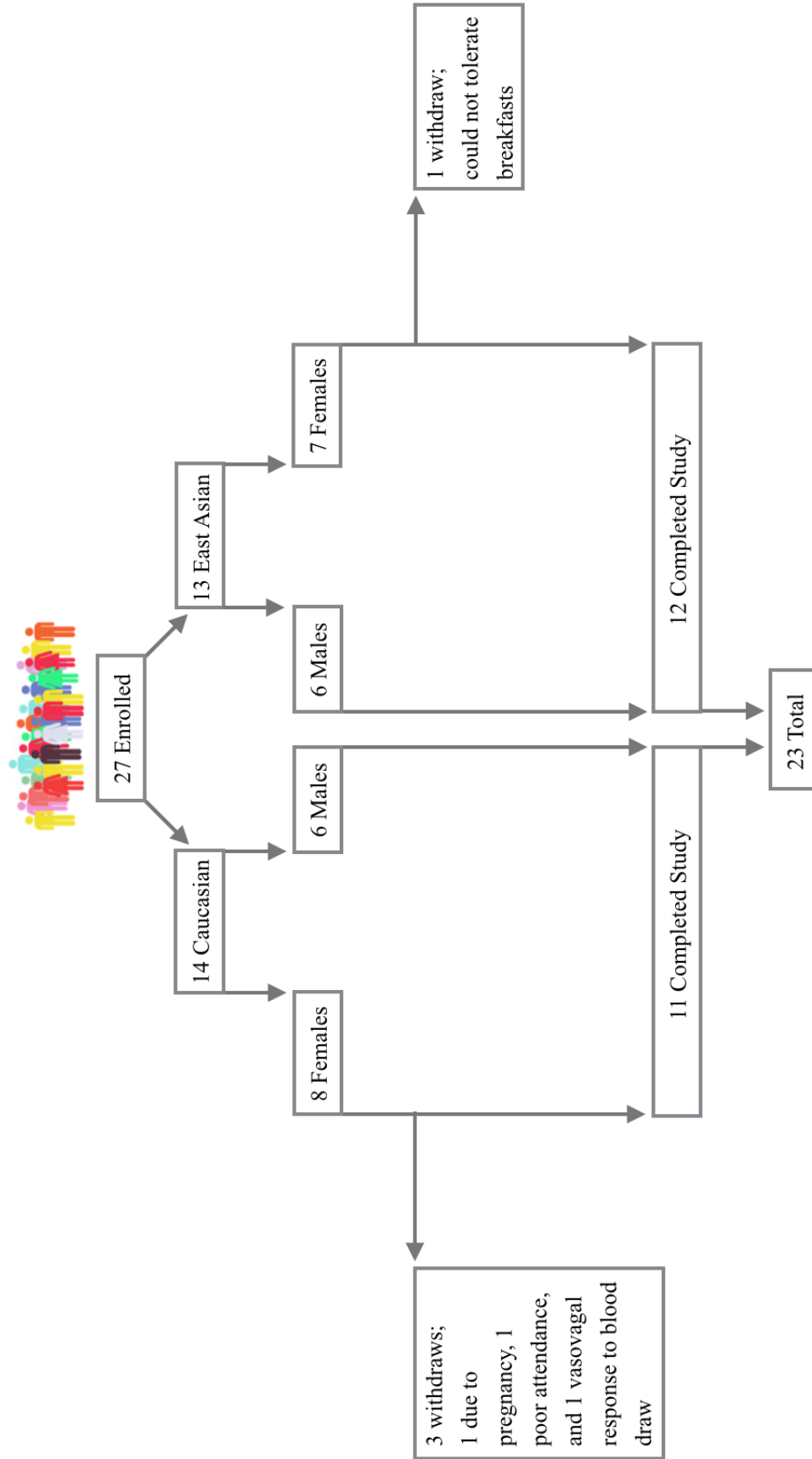


Figure 7. Recruitment tree for Human Pharmacokinetic Study.

Table 5. Demographic characteristics of healthy Caucasian subjects.

Males (n=6)								
Volunteer ID	Age, y	Weight, kg	Height, m	BMI, kg/m ²	Lipoprotein Analyses (mmol/L)			
					TC	Triglyceride	HDL-C	LDL-C
ROSF 1	22	93.10	1.79	29.06	5.96	3.11	1.17	3.38
ROSF 3	28	103.00	1.85	30.09	5.39	1.48	1.44	3.28
ROSF 5	59	80.10	1.77	25.71	5.48	2.18	1.28	3.21
ROSF 7	23	77.20	1.76	25.06	4.61	1.09	1.45	2.66
ROSF 14	22	77.50	1.74	25.75	2.99	1.26	1.32	1.10
ROSF 15	21	74.10	1.73	24.76	4.76	0.53	1.52	3.00
Average	29.17	84.17	1.77	26.74	4.87	1.61	1.36	2.77
SD	14.82	11.36	0.04	2.25	1.04	0.91	0.13	0.86
Females (n=5)								
Volunteer ID	Age, y	Weight, kg	Height, m	BMI, kg/m ²	Lipoprotein Analyses (mmol/L)			
					TC	Triglyceride	HDL-C	LDL-C
ROSF 2	23	78.00	1.69	27.31	4.60	1.44	1.98	1.97
ROSF 4	25	63.50	1.65	23.30	5.55	0.54	2.79	2.51
ROSF 11	21	94.00	1.75	30.69	3.95	0.69	1.60	2.04
ROSF 12	21	72.30	1.80	22.31	4.79	0.74	1.88	2.57
ROSF 13	22	62.20	1.72	21.02	4.81	1.08	1.62	2.70
Average	22.40	74.00	1.72	24.93	4.74	0.90	1.97	2.36
SD	1.67	12.93	0.06	3.99	0.57	0.36	0.48	0.33
Total Averag	26.09	79.55	1.75	25.92	4.81	1.29	1.64	2.58
Total SD	11.11	12.63	0.05	3.13	0.82	0.78	0.45	0.68

Abbreviations: SD, standard deviation; BMI, body mass index; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Table 6. Demographic characteristics of healthy East Asian subjects.

Males (n=6)								
Volunteer ID	Age, y	Weight, kg	Height, m	BMI, kg/m ²	Lipoprotein Analyses (mmol/L)			
					TC	Triglyceride	HDL-C	LDL-C
ROSF 17	22	84.50	1.74	27.91	4.31	1.54	1.33	2.28
ROSF 18	21	71.20	1.69	24.93	4.89	2.32	1.21	2.63
ROSF 19	22	89.90	1.78	28.37	4.29	0.84	1.51	2.40
ROSF 20	21	68.90	1.76	22.24	3.95	0.42	1.85	1.91
ROSF 21	21	70.50	1.62	26.86	3.44	0.62	1.34	1.82
ROSF 27	20	59.00	1.60	23.05	4.11	0.55	1.69	2.17
Average	21.17	74.00	1.70	25.56	4.17	1.05	1.49	2.20
SD	0.75	11.26	0.07	2.56	0.48	0.74	0.24	0.30
Females (n=6)								
Volunteer ID	Age, y	Weight, kg	Height, m	BMI, kg/m ²	Lipoprotein Analyses (mmol/L)			
					TC	Triglyceride	HDL-C	LDL-C
ROSF 16	21	43.60	1.59	17.25	3.78	0.50	2.00	1.55
ROSF 22	21	47.50	1.68	16.83	3.61	0.71	1.92	1.37
ROSF 23	22	58.00	1.60	22.66	4.30	0.79	1.29	2.65
ROSF 24	21	47.20	1.60	18.44	4.41	0.70	1.48	2.61
ROSF 26	20	44.50	1.47	20.59	3.97	0.64	1.83	1.85
ROSF 28	23	61.60	1.55	25.64	3.79	0.97	1.68	1.67
Average	21.33	50.40	1.58	20.23	3.98	0.72	1.70	1.95
SD	1.03	7.52	0.07	3.43	0.32	0.16	0.27	0.55
Total Average	21.25	62.20	1.64	22.90	4.07	0.88	1.59	2.08
Total SD	0.87	15.34	0.09	4.01	0.40	0.54	0.27	0.44

Abbreviations: SD, standard deviation; BMI, body mass index; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

4.1.2. Pharmacokinetic Analysis

Although rosuvastatin may be taken with or without food, recent work suggests that taking rosuvastatin with food may alter its plasma levels (Li *et al.*, 2009; Baek *et al.*, 2013). To elucidate the effect of concomitant food administration, and type of meal administered on the oral pharmacokinetics of rosuvastatin, we performed a prospective, randomized, cross-over study in healthy Caucasian and East Asian volunteers. Volunteers completed three separate study days on which they received 10 mg of rosuvastatin without food, with a low-fat meal, or with a high-fat meal; blood samples were collected over 10 hours and subsequently analyzed by LC-MS/MS. Caucasian (n=11) and East Asian (n=12) cohorts were analyzed together as a complete cohort (n=23) and separately to investigate ethnicity related differences.

Within both the Caucasian and East Asian subsets, large inter-individual variation was observed with regards to the plasma rosuvastatin concentration vs. time curves (**Figures 8A & B**). Within the Caucasian cohort, the coefficient of variation was approximately 19% for both C_{\max} and AUC_{0-10} values when rosuvastatin was administered in a fasted state (**Figure 8Ai**). Greater variation was observed in both fed states with approximately 45% and 43% variation in C_{\max} , and 37% and 31% variation in AUC_{0-10} within the low-fat and high-fat states, respectively (**Figures 8Aii & iii**). Within the East Asian cohort, fasting variation in C_{\max} and AUC_{0-10} were approximately 4-fold larger than corresponding values observed within the Caucasian subset (**Figure 8Bi**). Variation within the fed states were also quite high for the East Asian cohort, with approximately 80% and 60% variation in C_{\max} , and 87% and 63% variation in AUC_{0-10} within the low-fat and high-fat states, respectively (**Figures 8Bii & iii**). These data suggest that extent of variation in rosuvastatin levels may be dependent on ethnic differences.

Similar to previous reports, we observed a mean T_{\max} for rosuvastatin around 4 hours within the Caucasian cohort (DeGorter *et al.*, 2012b) (**Table 7**). The time to maximum concentration of rosuvastatin remained consistent throughout the fasted and fed states. Plasma rosuvastatin concentrations from 0 to 10 hours post dose for healthy Caucasian

individuals are displayed in **Figure 9A**. Within the Caucasian subset of volunteers, plasma rosuvastatin C_{\max} , AUC_{0-10} , and $AUC_{0-\infty}$, were significantly higher ($p < 0.001$, $p < 0.0001$, and $P < 0.0001$ respectively) when rosuvastatin was administered without food compared to administration with a low-fat meal (**Table 7, Figure 9B**). Similarly, rosuvastatin C_{\max} , AUC_{0-10} , and $AUC_{0-\infty}$, were significantly higher ($p < 0.05$, $p < 0.001$, and $p < 0.001$ respectively) when rosuvastatin was administered without food compared to administration with a high-fat meal (**Table 7, Figure 9B**). Lower systemic levels when rosuvastatin is administered with a meal suggests that food either impairs intestinal absorption or augments hepatic clearance of rosuvastatin. No significant differences in plasma rosuvastatin levels were observed between the low-fat and high-fat states (**Table 7, Figures 9A & B**).

Within the East Asian cohort, mean T_{\max} was approximately 1.5 and 1.4-fold longer when rosuvastatin was administered with a low-fat and high-fat meal, respectively, when compared to administration without food ($p < 0.05$ for both; **Table 7**). This indicates that within this population, the presence of food delayed the intestinal absorption of rosuvastatin. No significant differences were found in plasma C_{\max} , AUC_{0-10} , or $AUC_{0-\infty}$, among the fasting, low-fat, or high-fat states (**Table 7, Figures 9C & D**). However, plasma rosuvastatin levels (C_{\max} , AUC_{0-10} , and $AUC_{0-\infty}$) were approximately 1.8 and 1.4-fold lower when rosuvastatin was administered with a low-fat and a high-fat meal, respectively, when compared to levels attained under fasted conditions (**Table 7, Figures 9C & D**). This suggests that within this population, a food effect may be masked by large inter-individual variability.

Within the combined dataset (including both Caucasian and East Asian volunteers), C_{\max} , AUC_{0-10} , and $AUC_{0-\infty}$, were significantly higher ($p < 0.01$, $p < 0.01$, and $p < 0.05$ respectively) when rosuvastatin was administered without food compared to administration with a low-fat meal (**Table 7, Figures 9E & F**). Additionally, T_{\max} was significantly longer ($p < 0.05$) when rosuvastatin was administered with a low-fat meal compared to a fasted administration (**Table 7**). No significant differences were observed when the pharmacokinetic parameters from the high-fat state were compared to those

observed in the fasting or low-fat states (**Table 7, Figure 9E & F**). Moreover, no sex-related differences in AUC_{0-10} , $AUC_{0-\infty}$, C_{\max} , or T_{\max} were found (data not shown), which is consistent with previous reports (Martin *et al.*, 2002).

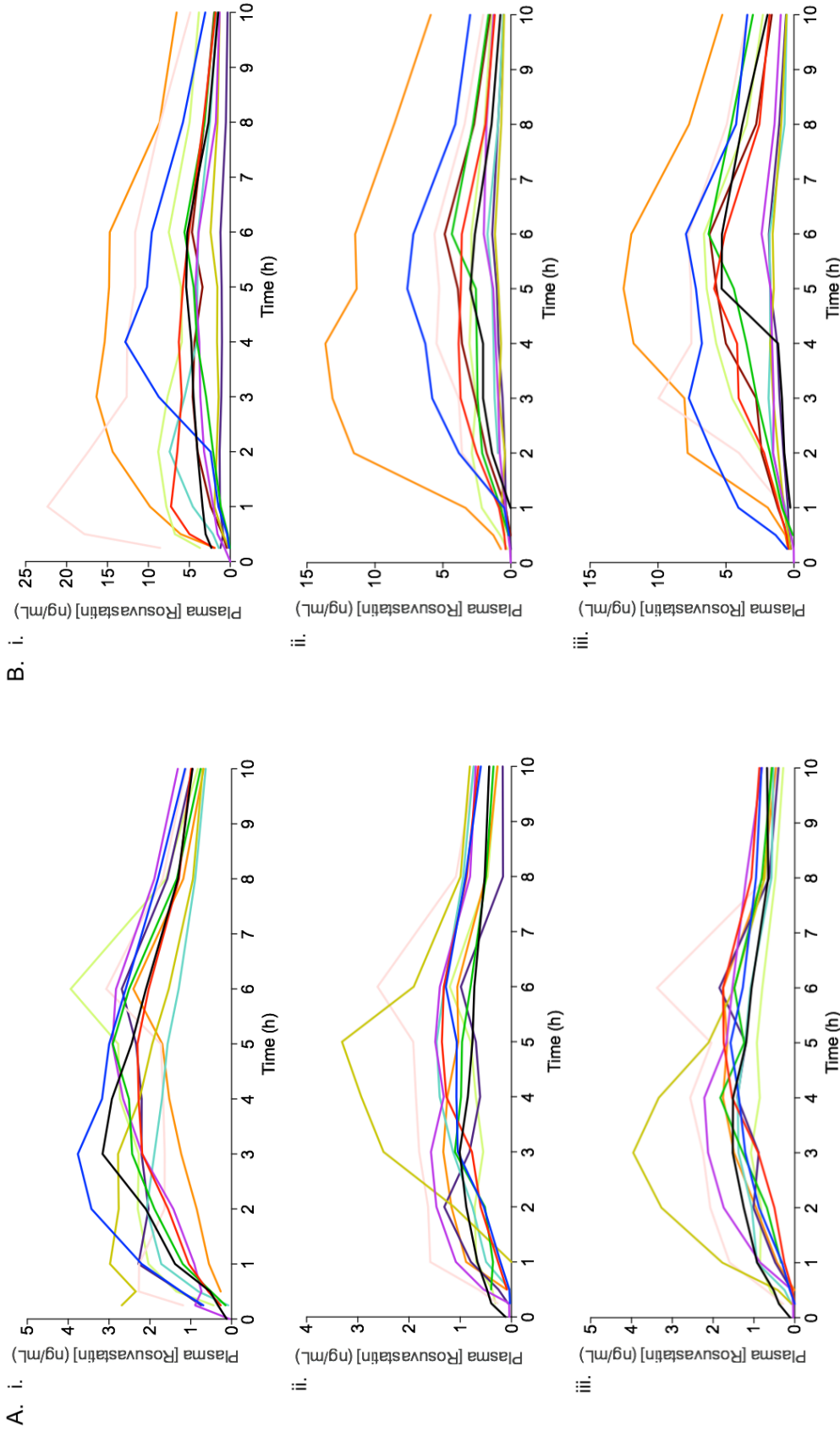


Figure 8. Inter-individual variation in plasma rosuvastatin concentration vs. time curves for (A) Caucasian and (B) East Asian individuals administered 10 mg of rosuvastatin (i) in a fasted state, (ii) with a low-fat meal, or (iii) with a high-fat meal. Plasma was collected from blood samples taken at 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, and 10 hours post dose. Plasma rosuvastatin concentrations were measured by LC-MS/MS. Note that within each subset of volunteers (Caucasian and East Asian), each colour represents the same individual for all three administration states.

Table 7. Pharmacokinetic parameters in healthy Caucasian and East Asian individuals administered 10 mg rosuvastatin in a fasted state, with a low-fat meal, or with a high-fat meal.

Caucasian Individuals (n=11)									
Parameter	Fasting vs. Low-fat	<i>p</i>	Fasting vs. High-fat	<i>p</i>	Low-fat vs. High-fat	<i>p</i>			
T _{max} (h)	4.4 (1.8)	4.3 (1.5)	0.989	4.4 (1.8)	4.4 (1.2)	> 0.999	4.3 (1.5)	4.4 (1.2)	0.989
C _{max} (ng/mL)	2.9 (0.6)	1.6 (0.7)	< 0.001	2.9 (0.6)	2.0 (0.9)	< 0.05	1.6 (0.7)	2.0 (0.9)	0.204
AUC ₀₋₁₀ (ng/mL*h)	18.3 (3.4)	9.4 (3.5)	< 0.0001	18.3 (3.4)	11.5 (3.6)	< 0.001	9.4 (3.5)	11.5 (3.6)	0.358
AUC _{0-∞} (ng/mL*h)	22.4 (4.2)	12.1 (4.2)	< 0.0001	22.4 (4.2)	14.5 (3.9)	< 0.001	12.1 (4.2)	14.5 (3.9)	0.343
East Asian Individuals (n=12)									
Parameter	Fasting vs. Low-fat	<i>p</i>	Fasting vs. High-fat	<i>p</i>	Low-fat vs. High-fat	<i>p</i>			
T _{max} (h)	3.6 (2.0)	5.3 (0.9)	< 0.05	3.6 (2.0)	5.1 (1.2)	< 0.05	5.3 (0.9)	5.1 (1.2)	0.903
C _{max} (ng/mL)	8.2 (6.1)	4.4 (3.5)	0.111	8.2 (6.1)	5.7 (3.4)	0.579	4.4 (3.5)	5.7 (3.4)	0.539
AUC ₀₋₁₀ (ng/mL*h)	50.6 (35.5)	27.4 (23.8)	0.081	50.6 (35.5)	33.8 (21.5)	0.427	27.4 (23.8)	33.8 (21.5)	0.601
AUC _{0-∞} (ng/mL*h)	62.6 (44.8)	36.1 (32.7)	0.132	62.6 (44.8)	43.4 (28.7)	0.484	36.1 (32.7)	43.4 (28.7)	0.691
Combined Dataset (n=23)									
Parameter	Fasting vs. Low-fat	<i>p</i>	Fasting vs. High-fat	<i>p</i>	Low-fat vs. High-fat	<i>p</i>			
T _{max} (h)	4.0 (1.9)	4.8 (1.3)	< 0.05	4.0 (1.9)	4.7 (1.2)	0.057	4.8 (1.3)	4.7 (1.2)	0.997
C _{max} (ng/mL)	5.7 (5.1)	3.0 (2.9)	< 0.01	5.7 (5.1)	4.0 (3.1)	0.209	3.0 (2.9)	4.0 (3.1)	0.374
AUC ₀₋₁₀ (ng/mL*h)	35.2 (30.1)	18.8 (19.3)	< 0.01	35.2 (30.1)	23.2 (19.1)	0.105	18.8 (19.3)	23.2 (19.1)	0.446
AUC _{0-∞} (ng/mL*h)	43.3 (37.8)	24.6 (26.3)	< 0.05	43.3 (37.8)	28.4 (25.2)	0.148	24.6 (26.3)	28.4 (25.2)	0.522

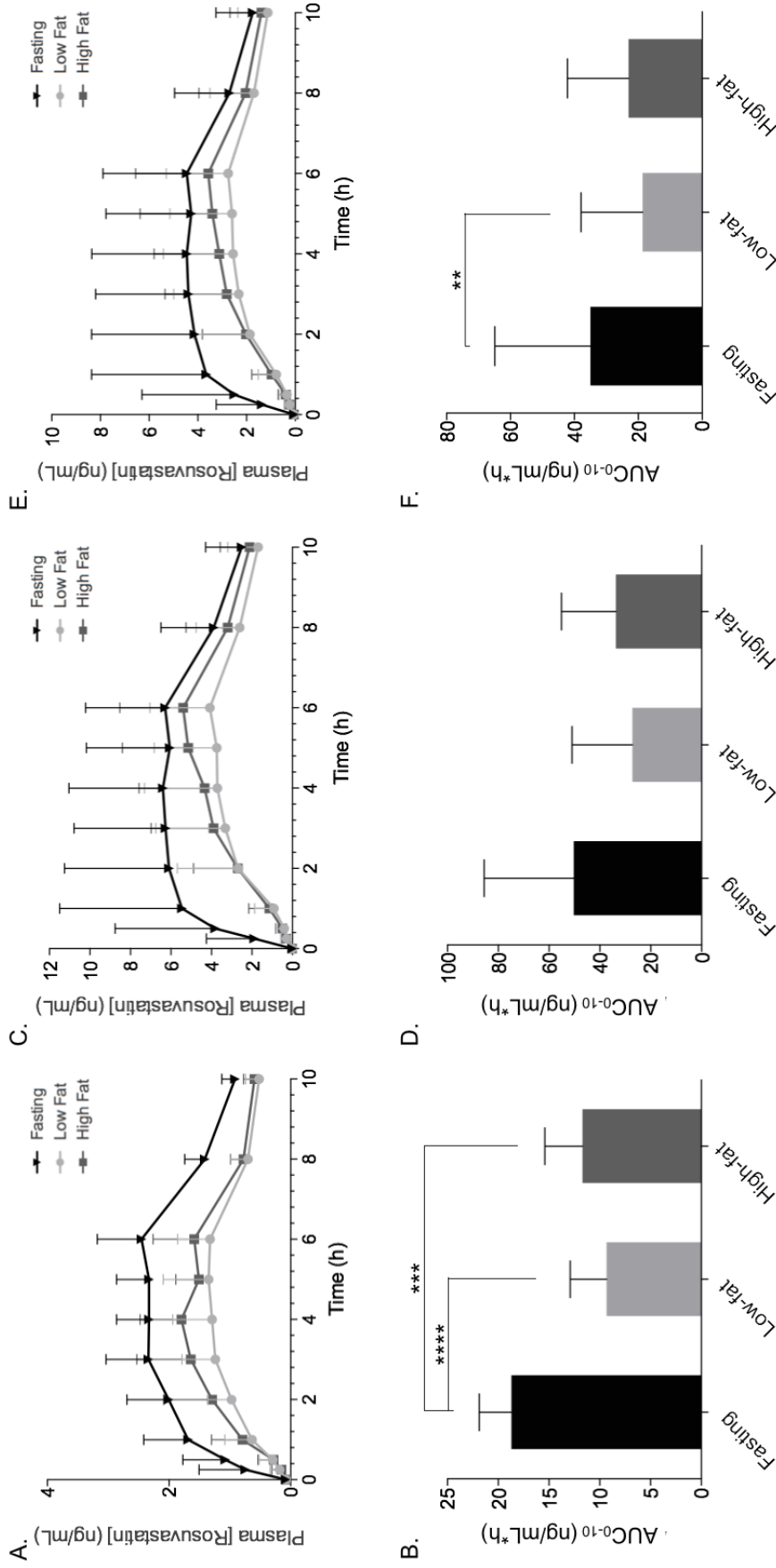


Figure 9. Rosuvastatin pharmacokinetic time curve followed by area under the curve (AUC₀₋₁₀) data for (A) & (B) healthy Caucasian volunteers (n=11), (C) & (D) healthy East Asian volunteers (n=12), and (E) & (F) the combined data set (n=23). Plasma was collected from blood samples taken at 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, and 10 hours post dose. Plasma rosuvastatin concentrations were measured by LC-MS/MS. Data are presented as mean + SD. Significance of the mean difference between administration states are depicted as ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

4.1.3. Pharmacogenetic Analysis

Previously, DeGorter *et al.* (2013) assessed the association of clinical PGx variables with the plasma rosuvastatin levels observed in a cohort of 130 rosuvastatin patients. After adjustment for age, ethnicity, body mass index, sex, dose, and time from last dose, Degorter *et al.* determined that plasma rosuvastatin concentrations were higher in individuals with variant alleles for *SLCO1B1* c.521T>C and/or *ABCG2* c.421C>A. Additionally, *SLCO1B1* c.388A>G and *ABCG2* c.34G>A have been associated with altered statin pharmacokinetics (Maeda *et al.*, 2006; Tornio *et al.*, 2015; Wan *et al.*, 2015). Here we analyzed these four genotypes within this population of healthy Caucasian (n=11) and East Asian volunteers (n=12).

Reported allele frequencies for *SLCO1B1* c.521T>C are around 15%, with similar frequencies detected within Caucasian and Asian populations. However, frequencies for *SLCO1B1* c.388A>G range from approximately 30-45% in Caucasian populations to 60-90% in Asian populations (Pasanen *et al.*, 2008). In our cohort, c.521T>C and c.388A>G frequencies were approximately 18% and 59% respectively for the Caucasian subset of volunteers. Within the East Asian subset, allelic frequencies for c.521T>C and c.388A>G were approximately 4% and 67%, respectively. Both *ABCG2* c.421C>A and c.34G>A have been found in Caucasian populations at frequencies <15%. In Asian populations, allelic frequencies for c.421C>A and c.34G>A are more common and have been reported as approximately 15-35% and 15-45%, respectively (Yasuda *et al.*, 2008). Within this population, c.421C>A was not detected in the Caucasian subset and had an allelic frequency of approximately 42% in the East Asian subset. Allele frequencies for c.34G>A were approximately 5% in the Caucasian subset and 38% in the East Asian subset of volunteers. Genotypes for all 23 volunteers are presented in **Table 8**.

To examine the impact of genotype on maximum rosuvastatin concentration and systemic exposure within this population, we compared plasma C_{\max} and $AUC_{0-\infty}$ values between wild-type individuals and carriers of a particular variant allele. For this analysis, heterozygous and homozygous variant carriers were combined. Moreover, because food

appears to affect plasma rosuvastatin concentrations, analysis was carried out using C_{\max} and $AUC_{0-\infty}$ values measured when a 10 mg oral dose of rosuvastatin was given directly following a high-fat meal, a low-fat meal, and in the absence of food. Within the fasted state, carriers of *ABCG2* c.421A had a 3.6-fold higher mean C_{\max} and 3.5-fold higher mean $AUC_{0-\infty}$ when compared to non-carriers (**Figures 10A & B**). No significant differences between mean C_{\max} or $AUC_{0-\infty}$ were found between wild-type individuals and variant carriers of *SLCO1B1* c.521T>C, *SLCO1B1* c.388A>G, or *ABCG2* c.34G>A (**Figure 10**). Within the high-fat and low-fat states, carriers of *ABCG2* c.421A had an approximate 3-fold higher mean C_{\max} when compared to non-carriers (**Figures 11A & 12A**). Similarly, mean $AUC_{0-\infty}$ was approximately 4-fold greater for *ABCG2* c.421 variant carriers when compared to wild-type individuals within both fed states (**Figures 11B & 12B**). No significant differences between mean C_{\max} or $AUC_{0-\infty}$ were found between wild-type individuals and variant carriers of *SLCO1B1* c.521T>C, *SLCO1B1* c.388A>G, or *ABCG2* c.34G>A for either of the fed states (**Figures 11 & 12**).

Within this population, all *ABCG2* c.421C>A variant carriers were of East Asian descent. Moreover, rosuvastatin C_{\max} and $AUC_{0-\infty}$ levels were, on average, higher within the East Asian cohort when compared to levels found within the Caucasian cohort. Therefore, to ensure that the correlation between *ABCG2* c.421A and plasma rosuvastatin levels was not biased by ethnicity differences, *ABCG2* c.421C>A was assessed in the East Asian cohort separate from the Caucasian cohort. Within the fasted state, mean C_{\max} was approximately 2-fold higher and mean $AUC_{0-\infty}$ was approximately 3-fold higher in variant-allele carriers when compared to non-carriers; however, these differences were not significant (**Figure 13A**). Within the low-fat state, variant carriers had significantly higher mean C_{\max} ($p=0.008$) and $AUC_{0-\infty}$ ($p=0.004$) when compared to non-carriers (**Figure 13B**). Similarly, mean C_{\max} ($p=0.008$) and $AUC_{0-\infty}$ ($p=0.008$) were significantly higher for variant carriers when compared to wild-type individuals (**Figure 13C**).

Table 8. Genotypes of healthy Caucasian and East Asian volunteers.

Caucasian Individuals (n=11)				
Volunteer ID	<i>SLCO1B1</i> Genotypes		<i>ABCG2</i> Genotypes	
	388A>G (*1b)	521T>C (*5)	421C>A	34G>A
ROSF 1	G/G	T/C	C/C	G/G
ROSF 2	G/G	T/T	C/C	G/G
ROSF 3	A/A	T/T	C/C	G/G
ROSF 4	G/G	T/T	C/C	G/A
ROSF 5	A/A	T/T	C/C	G/G
ROSF 7	A/G	T/C	C/C	G/G
ROSF 11	A/G	T/C	C/C	G/G
ROSF 12	G/G	T/C	C/C	G/G
ROSF 13	A/A	T/T	C/C	G/G
ROSF 14	G/G	T/T	C/C	G/G
ROSF 15	A/G	T/T	C/C	G/G
Total Carriers	8	4	0	1
East Asian Individuals (n=12)				
Volunteer ID	<i>SLCO1B1</i> Genotypes		<i>ABCG2</i> Genotypes	
	388A>G (*1b)	521T>C (*5)	421C>A	34G>A
ROSF 16	G/G	T/T	C/C	A/A
ROSF 17	G/G	T/C	C/A	G/G
ROSF 18	G/G	T/T	C/A	G/G
ROSF 19	G/G	T/T	C/A	G/G
ROSF 20	G/G	T/T	C/A	G/A
ROSF 21	A/A	T/T	C/C	G/A
ROSF 22	A/A	T/T	C/C	G/A
ROSF 23	A/G	T/T	C/A	G/A
ROSF 24	A/G	T/T	C/C	A/A
ROSF 26	G/G	T/T	A/A	G/G
ROSF 27	A/G	T/T	C/A	G/A
ROSF 28	A/G	T/T	A/A	G/G
Total Carriers	10	1	8	7

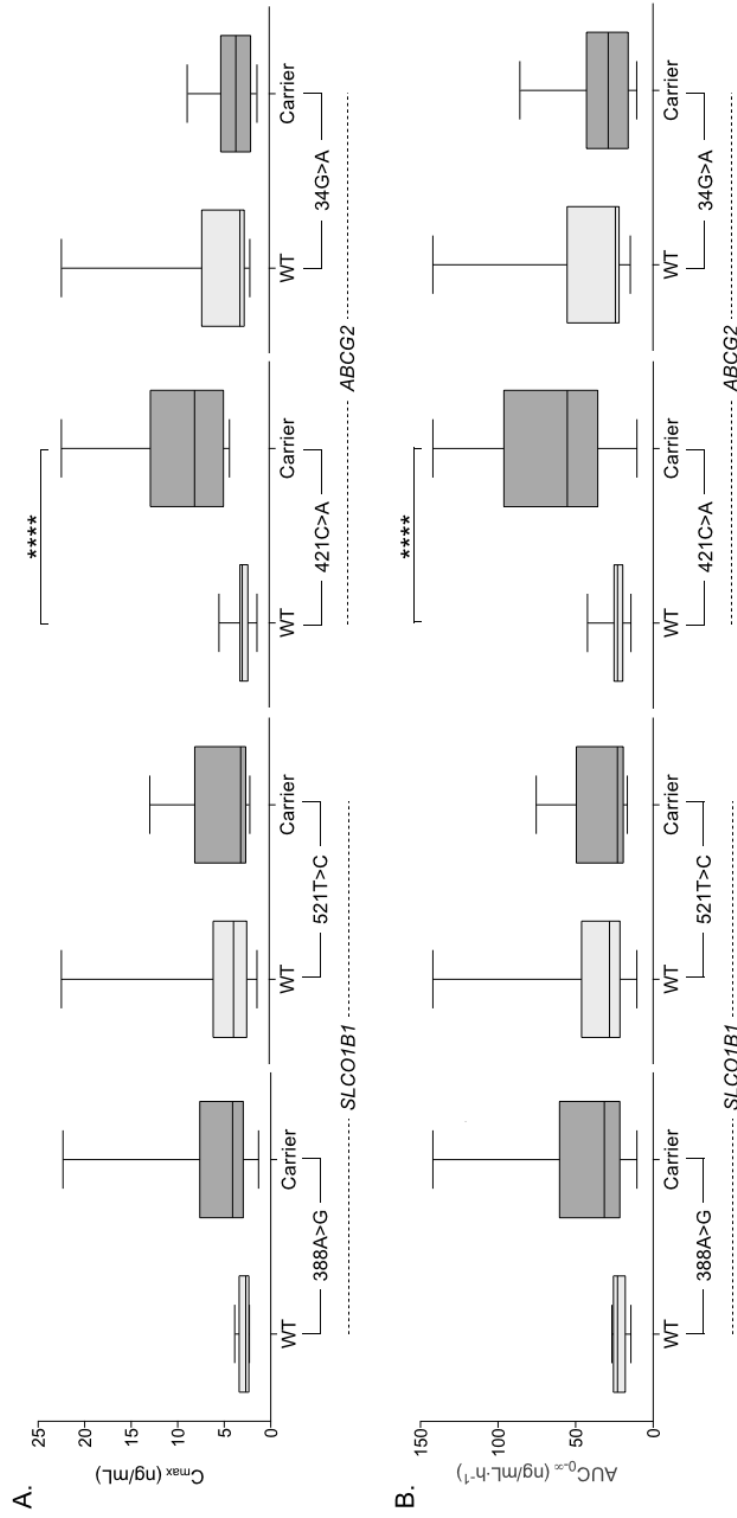


Figure 10. Effect of *SLCO1B1* c.388A>G and c.521T>C genotypes and *ABCG2* c.421C>A and c.34G>A genotypes on rosuvastatin (A) maximum concentration (C_{max}) and (B) area under the plasma concentration vs. time curve extrapolated out to infinity ($AUC_{0-\infty}$) in healthy Caucasian and East Asian (n=23) individuals administered 10 mg oral rosuvastatin without food. Top and bottom of the boxes represent the 25th and 75th percentile, respectively; the line between these represents the median. The whiskers depict the 5th and 95th percentile. Significance of the mean difference between variant carriers and wild-type (WT) individuals is depicted as ** $p < 0.0001$.**

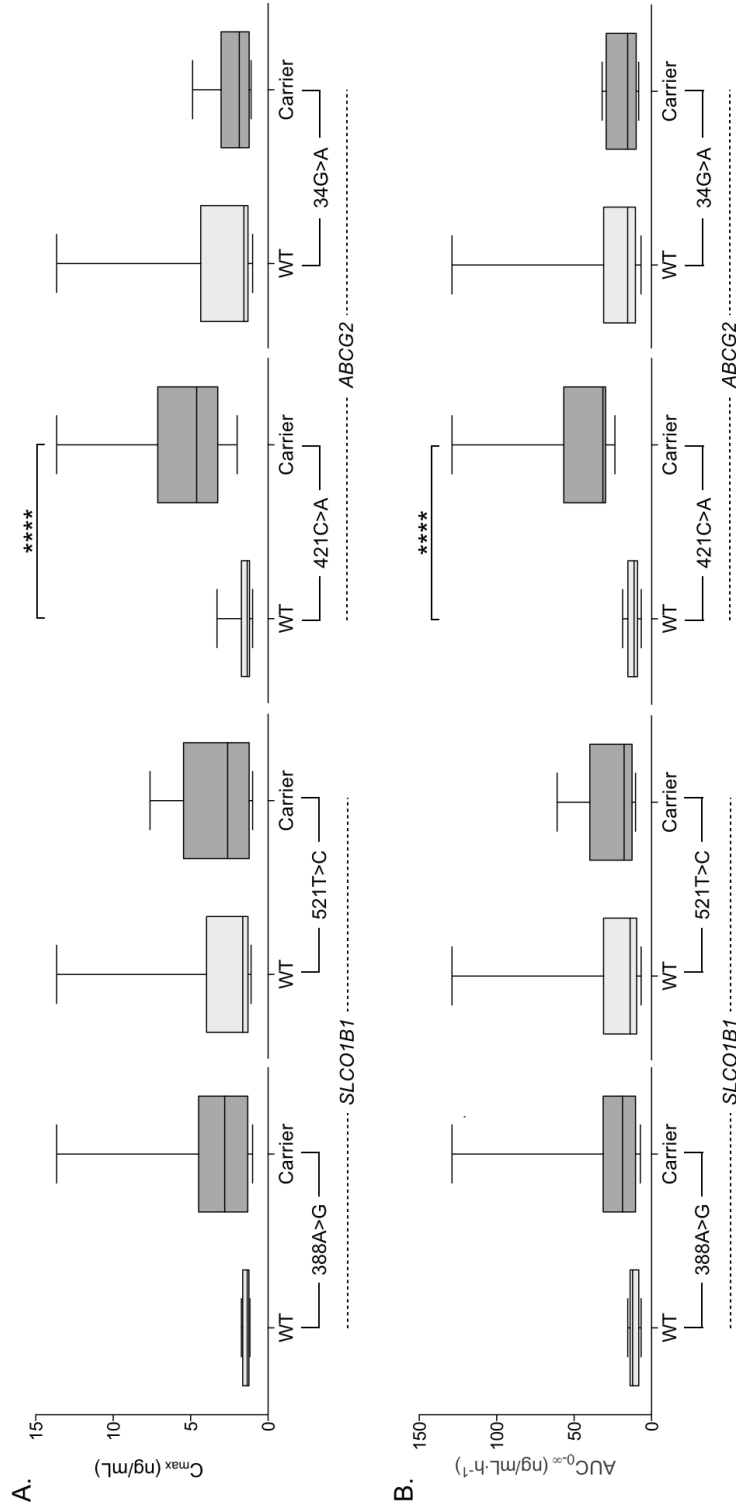


Figure 11. Effect of *SLCO1B1* c.388A>G and c.521T>C genotypes and *ABCG2* c.421C>A and c.34G>A genotypes on rosuvastatin (A) maximum concentration (C_{max}) and (B) area under the plasma concentration vs. time curve extrapolated out to infinity ($AUC_{0-\infty}$) in healthy Caucasian and East Asian (n=23) individuals administered 10 mg oral rosuvastatin following a low-fat meal. Top and bottom of the boxes represent the 25th and 75th percentile, respectively; the line between these represents the median. The whiskers depict the 5th and 95th percentile. Significance of the mean difference between variant carriers and wild-type (WT) individuals is depicted as ** $p < 0.0001$.**

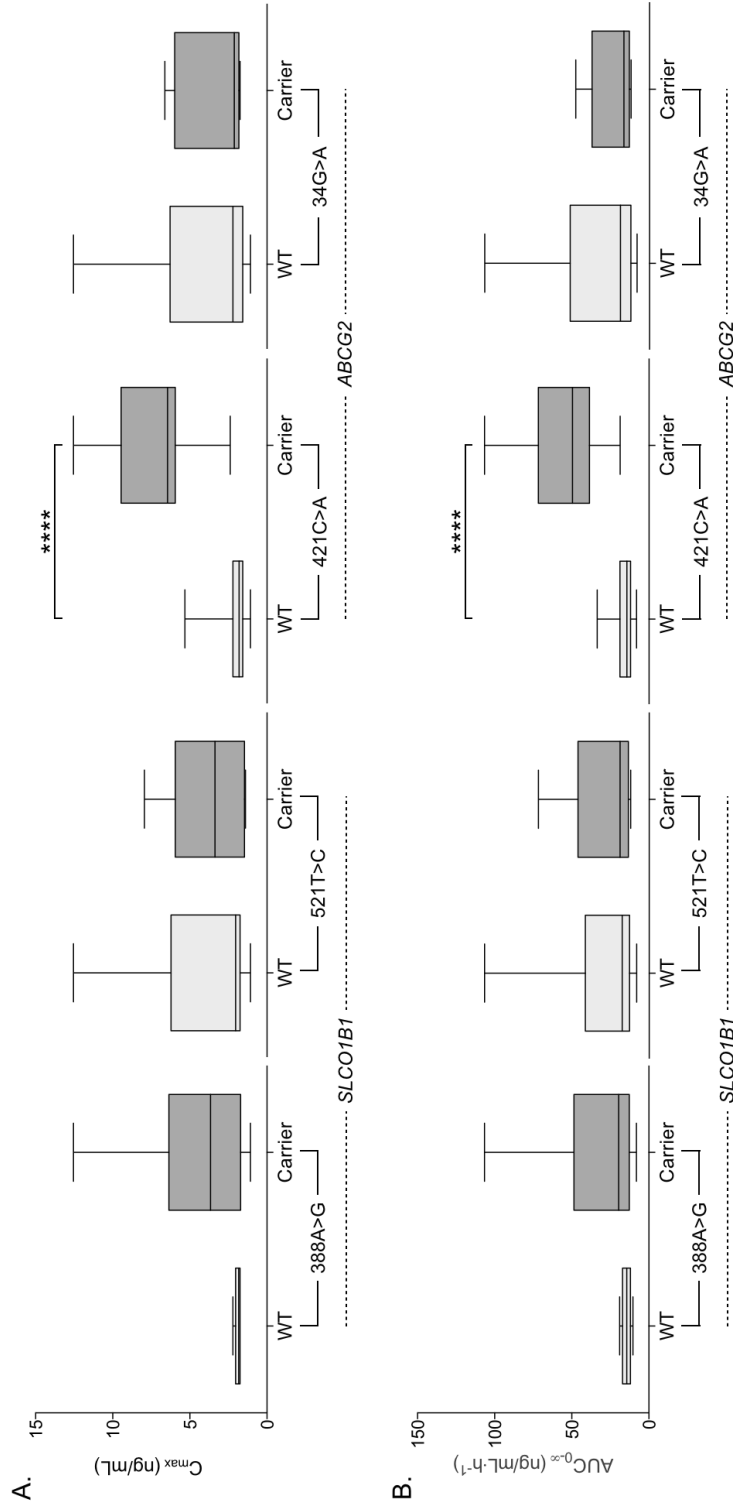


Figure 12. Effect of *SLCO1B1* c.388A>G and c.521T>C genotypes and *ABCG2* c.421C>A and c.34G>A genotypes on rosuvastatin (A) maximum concentration (C_{max}) and (B) area under the plasma concentration vs. time curve extrapolated out to infinity ($AUC_{0-\infty}$) in healthy Caucasian and East Asian (n=23) individuals administered 10 mg oral rosuvastatin following a high-fat meal. Top and bottom of the boxes represent the 25th and 75th percentile, respectively; the line between these represents the median. The whiskers depict the 5th and 95th percentile. Significance of the mean difference between variant carriers and wild-type (WT) individuals is depicted as ** $p < 0.0001$.**

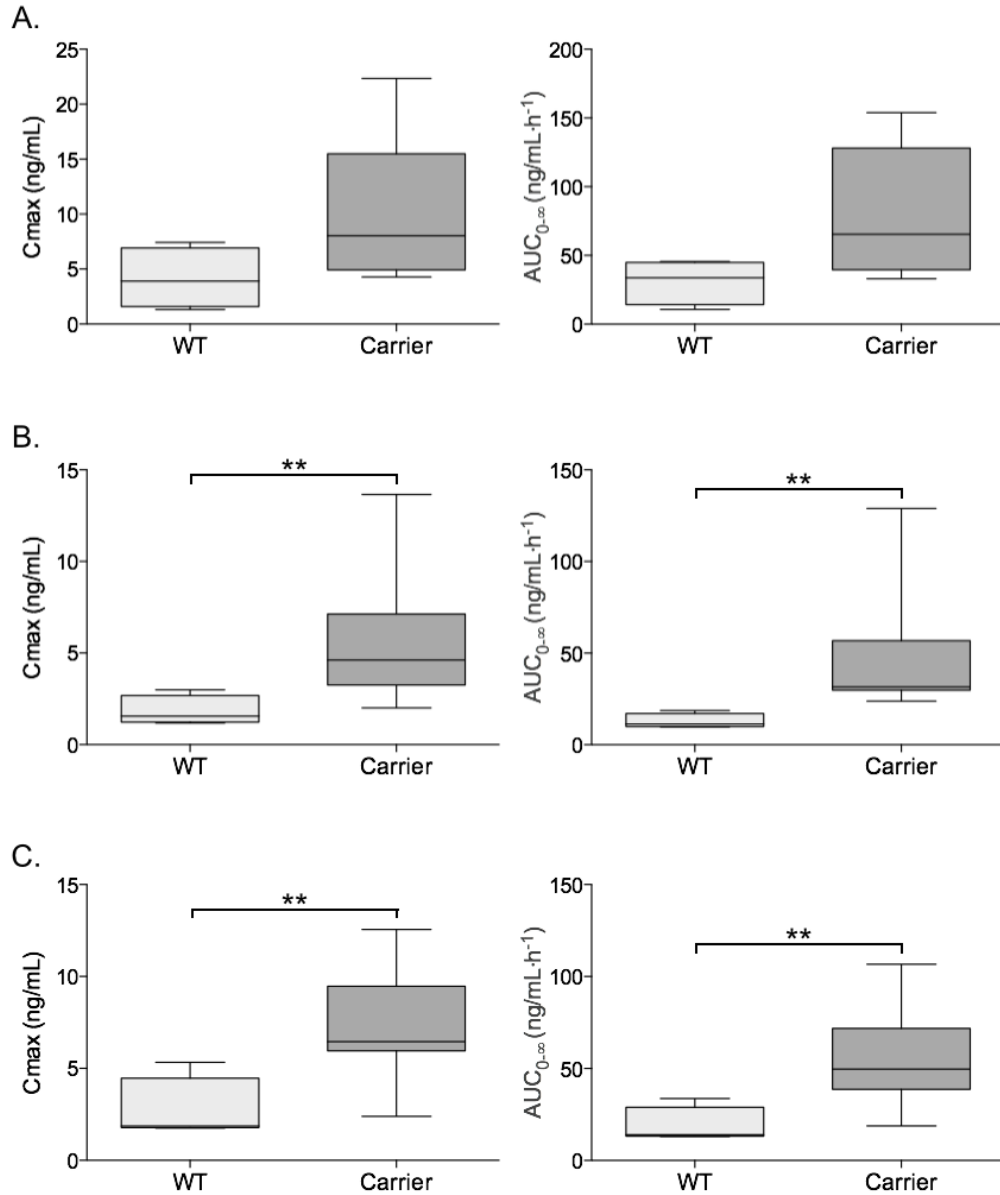


Figure 13. Effect of *ABCG2* c.421C>A on rosuvastatin maximum concentration (C_{max}) and B. area under the plasma concentration-time curve extrapolated out to infinity ($AUC_{0-\infty}$) in healthy East Asian (n=12) individuals administered 10 mg oral rosuvastatin (A) without food, (B) with a low-fat meal, and (C) with a high-fat meal. Top and bottom of the boxes represent the 25th and 75th percentile, respectively; the line between these represents the median. The whiskers depict the 5th and 95th percentile. Significance of the mean difference between variant carriers (n=8) and wild-type (WT; n=4) individuals is depicted as ** $p < 0.01$.

4.2. Retrospective Analysis of Patient Data

To investigate the effect of concomitant food administration with rosuvastatin dose on the lipid profiles of statin patients, we analyzed data from 157 previously recruited individuals from the LHSC Lipid Clinic. Upon enrolment, a blood sample was collected and the daily dose of rosuvastatin, time of sample collection, and time since last dose were recorded (DeGorter *et al.*, 2013). Patients that reported taking their last rosuvastatin dose between 5-9 am (inclusive) or 5-7 pm (inclusive) were presumed to have taken the medication with food (n=75). Patients that reported taking their last rosuvastatin dose at or after 8 pm were presumed to have taken the medication without food (n=82). Moreover, because patients tend to take their medications at the same time every day, we presumed that this data would reflect the daily administration habits of these patients.

Within both populations, the majority of patients were Caucasian males. Median age (range) and BMI \pm SD for the cohort of individuals presumed to take their rosuvastatin with food were 59.0 (19-80) years and 29.9 ± 5.9 kg/m² respectively. In the cohort of individuals presumed to take their rosuvastatin without food the median age (range) and BMI \pm SD were 58.5 (23-90) years and 30.7 ± 7.6 kg/m² respectively. The average number of concomitant medications \pm SD taken by patients presumed to take their rosuvastatin with and without food were 6.4 ± 3.2 and 6.3 ± 3.5 respectively. Further details regarding population characteristics can be found in **Table 9**.

Rosuvastatin targets the liver and effectively reduces plasma concentrations of LDL-C. Patients of the LHSC Lipid Clinic have their rosuvastatin doses adjusted so that their LDL-C values fall within a defined range. Within this study population, the average dose of rosuvastatin was not significantly different between individuals presumed to take rosuvastatin with or without food (**Figure 14**). This indicates that in this population, taking rosuvastatin with or without food did not affect its capacity to lower LDL-C.

We further divided the cohorts by dose and compared plasma concentrations of LDL-C, total cholesterol, and lathosterol, a cholesterol biosynthesis intermediate. Taking rosuvastatin with or without food did not significantly affect average LDL-C or lathosterol concentrations for individuals taking a daily 5 mg, 10 mg, 20 mg, or 40 mg rosuvastatin dose (**Figure 15A** and **15C**). At a dose of 5 mg of rosuvastatin, total cholesterol was found to be significantly lower ($p=0.032$) in patients that took their dose with food compared to those that took their dose without food (**Figure 15B**). However, at 10 mg, 20 mg, and 40 mg, no significant differences in average total cholesterol were found between individuals presumed to take their rosuvastatin dose with or without food (**Figure 15B**). Together, these results indicate that taking rosuvastatin with or without food does not alter the amount of rosuvastatin that reaches the liver and thus does not affect the ability of rosuvastatin to inhibit endogenous cholesterol production.

Table 9. Characteristics of London Health Sciences Centre lipid clinic patients taking rosuvastatin once daily with or without food.

	With Food	Without Food
Number of patients	75	82
% Male	70.7	72.0
Age at enrolment (years)	56.9 (13.2)	56.7 (13.3)
% Caucasian	90.7	84.1
Body mass index (kg/m ²)	29.9 (5.9)	30.7 (7.6)
Number of concomitant medications	6.4 (3.2)	6.3 (3.5)
% of patients on		
5 mg	16.0	14.6
10 mg	34.7	26.8
20 mg	29.3	30.5
40 mg	18.7	25.6
Other	1.3	2.4

Data are presented as % or mean (SD).

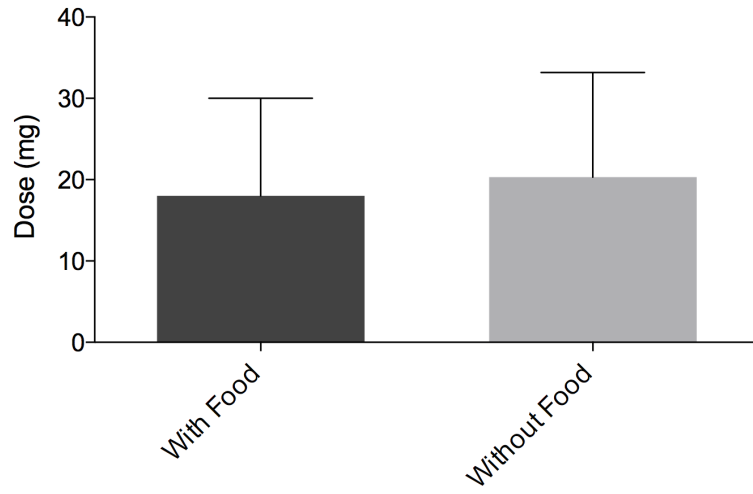


Figure 14. Average dose of rosuvastatin taken by London Health Sciences Centre lipid clinic patients presumed to take their dose with (n=75) or without (n=82) food. Data are presented as mean + SD.

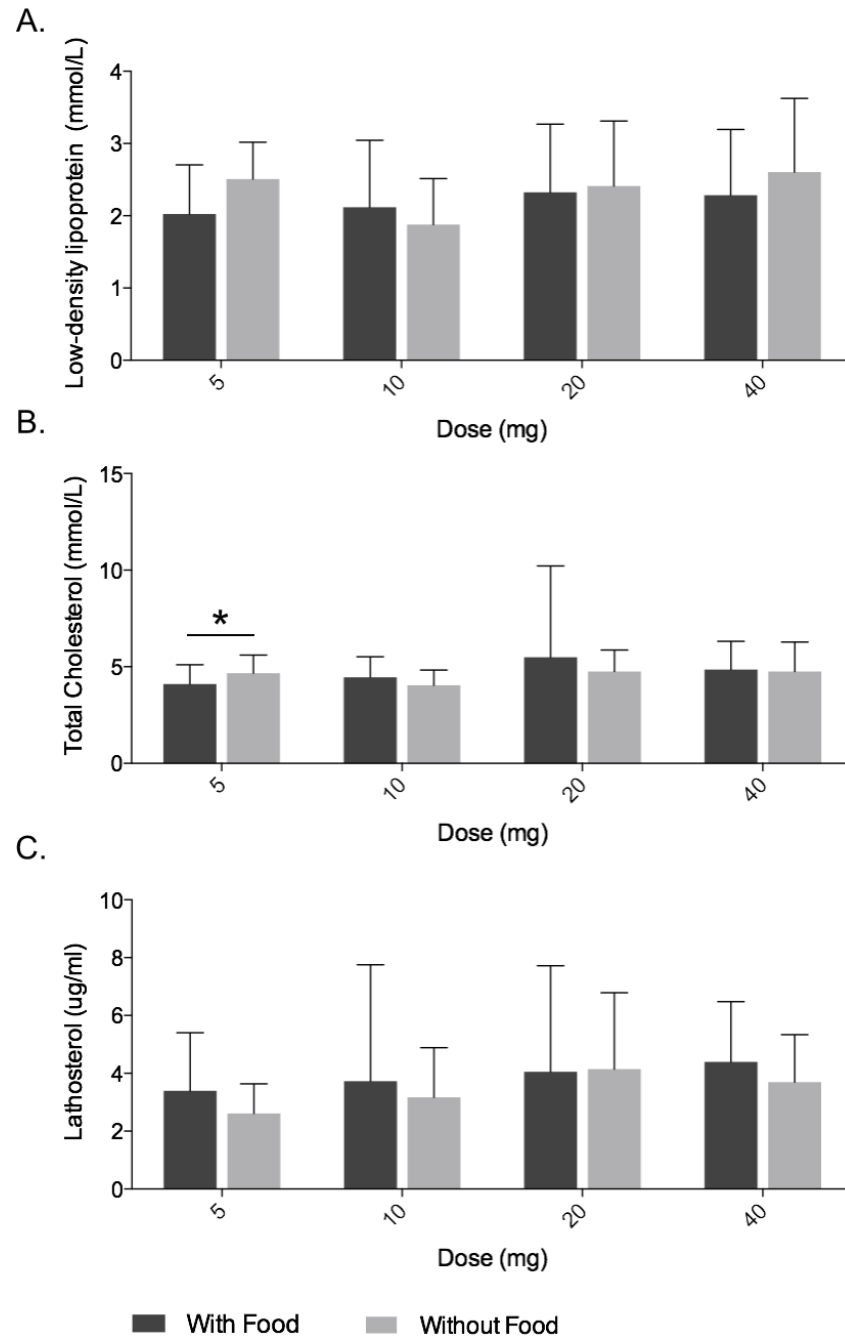


Figure 15. Average (A) low-density lipoprotein cholesterol (n=146), (B) total cholesterol (n=154), and (C) lathosterol (n=154) concentrations in London Health Sciences Centre lipid clinic patients presumed to take rosuvastatin with or without food. The data are divided by dose of daily rosuvastatin and are presented as mean + SD. Lipid parameter values were compared at each dose and significance is depicted by $*p<0.05$.

4.3. Mouse Pharmacokinetic Study

Taking rosuvastatin with food has been shown to result in lower drug plasma levels when compared to those achieved from a fasted administration (Li *et al.*, 2009; Baek *et al.*, 2013). This may be due to either decreased intestinal absorption or increased hepatic clearance of rosuvastatin in the presence of food. To further investigate a potential food effect, we performed a pharmacokinetic study whereby 10 mg/kg of rosuvastatin was administered via oral gavage to fed and fasted wild-type C57BL/6 mice. Previous data from our laboratory examining rosuvastatin pharmacokinetics after oral administration in wild-type C57BL/6 mice showed that the mean plasma $AUC_{0-\infty}$ was approximately 127.6 ng*h/mL with a standard deviation of 23.2 (n=6; Knauer, 2012). Using this data, we calculated that a sample size of n=5 per group would provide 80% power to observe a 30% difference in plasma rosuvastatin levels at 95% confidence. One mouse was excluded as an outlier because its C_{max} and AUC values were 9-fold greater than the average values within the same group. Data from n=4 fed mice and n=5 fasted mice were analyzed.

Comparable to earlier reports, we found the oral absorption of rosuvastatin to be quite rapid (Peng *et al.*, 2009; Knauer, 2012). The highest rosuvastatin plasma concentrations were observed at the the first time point (7.5 min) for both fed and fasted mice (**Figure 16**). Half-life of rosuvastatin was not significantly different between fed and fasted mice (**Table 10**). Analysis of mean values for C_{max} , AUC_{0-2} , and $AUC_{0-\infty}$ revealed an approximate 2.7, 2.8, and 2.4 respective fold increase when rosuvastatin was given under fasted conditions compared to a fed administration. However, due to large inter-mouse variation, no significant differences were found in C_{max} , AUC_{0-2} , or $AUC_{0-\infty}$ between fed and fasted mice (**Table 10**). These results would suggest that food does not significantly affect the oral pharmacokinetics of rosuvastatin in this mouse model; however, it is possible that large inter-mouse variability is masking an underlying effect.

The liver-to-plasma concentration ratio of a medication is a sensitive marker of its hepatic uptake. Therefore, to investigate hepatic transport of rosuvastatin in response to food

intake, we measured the terminal plasma concentration, liver concentration, and liver-to-plasma concentration ratio of rosuvastatin in our fed and fasted mice. At the terminal time point (2 hours), the average plasma rosuvastatin concentration was significantly greater ($p=0.0159$) for fasted mice when compared to fed mice (**Figure 17A**). However, liver concentrations of rosuvastatin were not significantly different ($p=0.2857$) between fed and fasted mice (**Figure 17B**). Mean liver-to-plasma concentration ratio of rosuvastatin was 2.1-fold greater in fed mice when compared to fasted mice, however these values were not significantly different ($p=0.1905$; **Figure 17C**). Taken together, these results suggest that in this mouse model, hepatic uptake of rosuvastatin is not altered by the presence of food. However, higher 2 hour plasma concentrations in fasted mice and an approximate 2-fold increase in liver-to-plasma concentration ratio in fed mice when compared to fasted mice indicates that an effect may be masked by large inter-mouse variability.

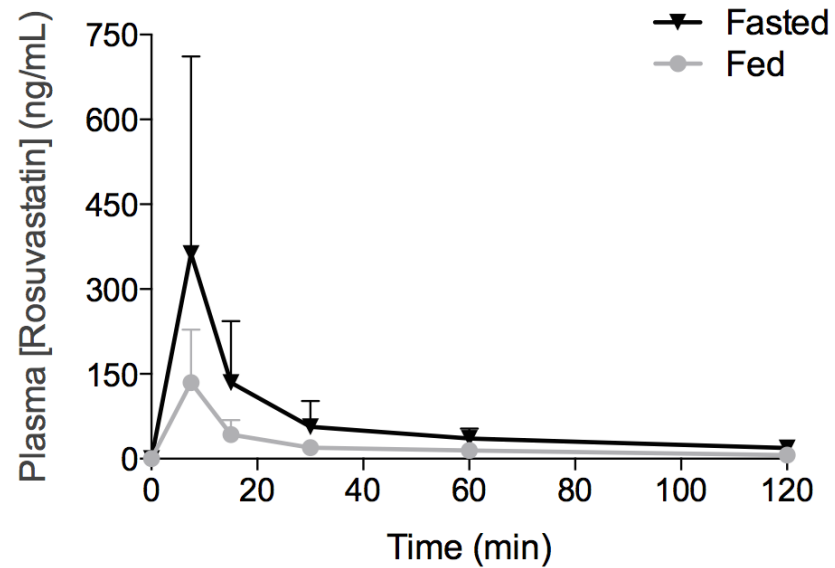


Figure 16. Plasma concentration-time curves of rosuvastatin in fed (n=4) and fasted (n=5) C57BL/6 mice after administration of 10 mg/kg rosuvastatin via oral gavage. Plasma was collected from blood samples taken at 7.5, 15, 30, 60, and 120 min post dose. Plasma rosuvastatin concentrations were measured by LC-MS/MS. Data are presented as mean + SD.

Table 10. Analysis of pharmacokinetic parameters in fed and fasted wild-type C57BL/6 mice after administration of 10 mg/kg rosuvastatin via oral gavage.

Parameter	Fasting (n=5)	Fed (n=4)	<i>p</i>
T _{1/2} (min)	48.5 (20.2)	91.6 (75.9)	0.7143
C _{max} (ng/mL)	362.6 (349.1)	134.6 (94.1)	0.3968
AUC ₀₋₂ (ng/mL*min)	7712.8 (5531.6)	2781.4 (1653.6)	0.0635
AUC _{0-∞} (ng/mL*min)	9097.7 (5665.3)	3764.2 (1331.3)	0.0635

Data are presented as mean (SD).

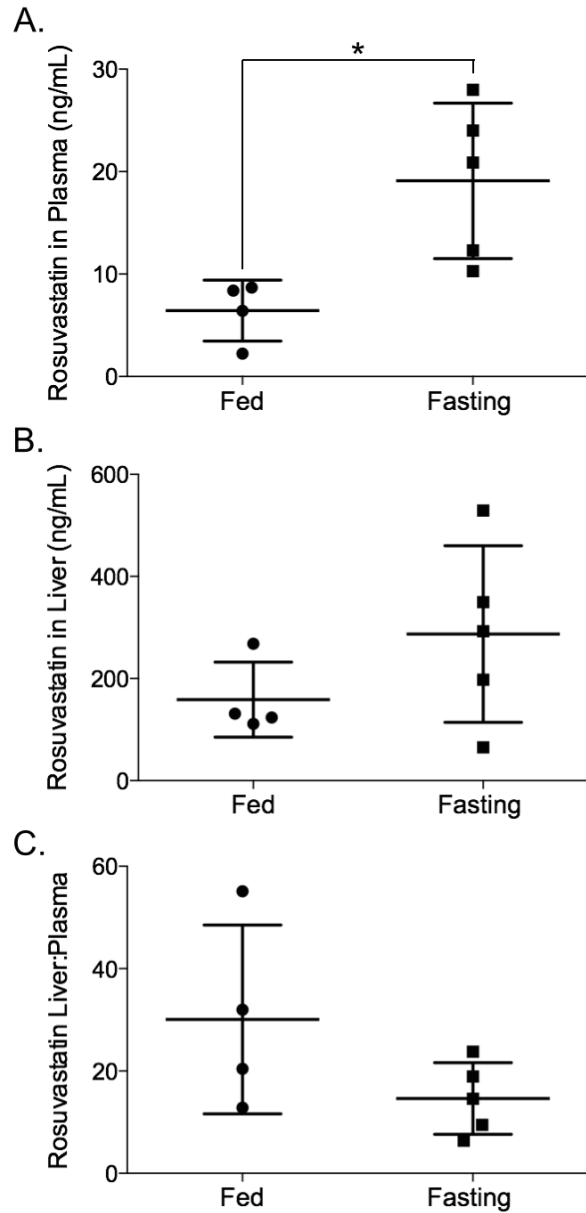


Figure 17. Rosuvastatin concentrations in (A) plasma, (B) liver, and (C) the liver-to-plasma concentration ratio in fed (n=4) and fasted (n=5) C57BL/6 mice 2 hours after a 10 mg/kg oral rosuvastatin dose. Plasma was collected from blood samples taken at 2 hours post dose. Livers were excised 2 hours post dose and later homogenized. Rosuvastatin concentrations in the plasma and liver homogenate samples were measured by LC-MS/MS. Data presented as mean with SD. Significance of the mean difference between fed and fasted mice is depicted by $*p < 0.05$.

5. SUMMARY & DISCUSSION

Statin medications are first-line pharmacotherapy for the treatment of hypercholesterolemia and prevention of cardiovascular disease, and an estimated 25.2 million Americans are currently receiving statin therapy (Pencina *et al.*, 2014). Statins function through targeted accumulation within the liver and inhibit the rate-limiting enzyme in cholesterol biosynthesis, HMG-CoA reductase (Ho *et al.*, 2006). High circulating levels of statins have been associated with a number of statin-induced adverse events (Golomb & Evans, 2008). Indeed, muscle pain or weakness occurs in up to 10% of statin patients and is a frequent cause of discontinuation of statin therapy (Joy & Hegele, 2009; Abd & Jacobson, 2011). Observed inter-individual variation in plasma statin exposure in patients is associated, in part, with polymorphisms within hepatic uptake and efflux transporters such as OATP1B1 and the efflux transporter BCRP. However, given the 45-fold or higher variability in plasma statin levels between patients on the same daily dose (DeGorter *et al.*, 2013), it is clear that known genetic polymorphisms in statin disposition pathways do not adequately account for all variation in statin exposure, suggesting that additional pathways or mechanisms may be involved. Interestingly, taking medications with food has the potential to alter the physiochemical properties of the drug, and/or change the pharmacokinetic or pharmacodynamic profiles of the drug, thus leading to variation in plasma exposure (Singh, 1999).

Significant evidence indicates that plasma levels of commonly prescribed statins, such as pravastatin and atorvastatin, are lower when administered with food compared to a fasted administration (Pan *et al.*, 1993; Radulovic *et al.*, 1995). Currently, there is conflicting evidence regarding the effect of food on plasma rosuvastatin levels. The approval package for Crestor® (rosuvastatin calcium) suggests that food does not alter rosuvastatin systemic exposure (Center for Drug Evaluation and Research, 2012). However, a study performed in healthy Chinese subjects indicated a profound decrease in rosuvastatin plasma levels (both C_{max} and AUC) when rosuvastatin was administered with food (Li *et al.*, 2009).

A primary objective of this study was to investigate the effect of concomitantly administered meals (both high-fat and low-fat meals) on plasma rosuvastatin concentrations in healthy Canadian East Asian and Caucasian volunteers. We demonstrated for the first time that concomitant administration with food substantially reduced mean plasma rosuvastatin C_{max} and AUC in healthy Caucasian and East Asian subjects. We found that within healthy Caucasian individuals administering 10 mg oral rosuvastatin with a low-fat or high-fat meal resulted in an average reduction in mean plasma rosuvastatin levels (average of C_{max} , AUC_{0-10} , and $AUC_{0-\infty}$) of approximately 46% and 35%, respectively when compared to values observed under fasting conditions. We found similar reductions of approximately 45% and 31%, for the low-fat and high-fat states respectively, in an East Asian cohort. Similarly, within mice we observed considerably lower AUC values when 10 mg/kg rosuvastatin was administered orally to fed mice when compared to fasted mice. However, in part due to sample size and marked variation between mice, this difference was not found to be significant.

Previously, Li *et al.* (2009) found that administering 10 mg oral rosuvastatin to healthy Chinese subjects directly following a large meal resulted in >90% decrease in mean AUC and C_{max} values when compared to fasting values. Li *et al.* used generic rosuvastatin provided by DYNE PHARMA, a manufacturer in Shandong, China. Formulation differences between these tablets and the Crestor® tablets used in our study may account for the difference in the magnitude of the response to food. Moreover, the test meal used by Li *et al.* was larger than either of the treatment breakfasts used in our study and consisted of approximately 1046 kcal and 43 g of fat compared to 712 kcal and 37 g for the high-fat meal and 661 kcal and 15 g for the low-fat meal used in our study. Administration of a larger test meal may also help to explain the difference in the magnitude of the response to food between the two studies. Similar to Li *et al.*, we found that administration with food significantly increased time to maximum rosuvastatin concentration within our East Asian cohort. Interestingly, we did not observe this effect within our Caucasian cohort, suggesting ethnic differences in the response to food.

Although the overall percent reduction in rosuvastatin systemic exposure was similar between Caucasian and East Asian subjects.

Similar to Li *et al.*, we observed the overall effect of reduced rosuvastatin exposure when it is taken with food compared to administration without food. Li *et al.* concluded that rosuvastatin systemic exposure was likely lower when administered with food due to impaired intestinal absorption. However, lower systemic exposure could also be explained by increased hepatic uptake and clearance of rosuvastatin in the presence of food. To investigate the contribution of hepatic uptake of rosuvastatin in the presence of food, we administered 10 mg/kg of rosuvastatin to fed and fasted mice and then compared their plasma and liver drug concentrations 2 hours post dose. We observed significantly higher plasma rosuvastatin concentrations at the terminal time point in fasted mice when compared to fed mice. Moreover, we observed no significant difference in the liver concentrations of rosuvastatin between the fed and fasted mice. The liver-to-plasma concentration ratio of a medication is a sensitive marker of its hepatic uptake. Within mice, mean liver-to-plasma concentration ratio was approximately 2.1-fold higher in fed mice when compared to fasted mice administered oral rosuvastatin, although this difference was not significant. We observed rather large inter-mouse variability in rosuvastatin levels and are unable to confirm differences in hepatic uptake of rosuvastatin when it is administered to fed and fasted mice. Importantly, as this work was performed in mice, it may not be truly reflective of what occurs in humans.

Although the OATP family of transporters has been viewed as the principal mediators for the hepatic uptake of statins, previous data in our lab demonstrates that liver-specific NTCP may account for nearly one third of rosuvastatin uptake (Ho *et al.*, 2006). Increased expression or activity of NTCP would, therefore, aid in clearance of rosuvastatin from portal circulation, while lowering circulating statin concentrations. NTCP is a major transporter involved the enterohepatic recirculation of bile acids, and its expression and activity are highly regulated to allow for efficient management of a high bile-acid load associated with food ingestion (Anwer & Stieger, 2014). Since a larger bile-acid load accompanies the ingestion of a high-fat meal (Marciani *et al.*, 2013), we

hypothesized that plasma rosuvastatin levels would be lowest when a rosuvastatin dose was administered with a high-fat meal. Indeed, Baek *et al.* (2013) found that C_{\max} and AUC of rosuvastatin were significantly lower when a 10 mg oral dose was administered to dogs fed a high-fat meal when compared to those fed a low-fat meal prior to dosing. Interestingly, we did not observe any significant differences in C_{\max} or AUC values when rosuvastatin was administered following a high-fat compared to administration following a low-fat meal within our healthy human cohorts. This disparity may, in part, be explained by differences in gastrointestinal physiology and biochemistry and/or variability in expression of important hepatobiliary transporters between humans and dogs (Kararli, 1995; Wang *et al.*, 2015). Moreover, it is possible that the difference in fat content between the high-fat and low-fat breakfasts used in the present study was not large enough to detect a difference with respect to an effect on rosuvastatin pharmacokinetics in humans.

Efficacy of statin-mediated HMG-CoA reductase inhibition is often determined through measuring reductions in LDL-C or lathosterol, a late intermediate in cholesterol biosynthesis. Previous work has shown that LDL-C reductions were not significantly different in healthy volunteers following administration of 10 mg atorvastatin tablets with or without food for 15 days (Whitfield *et al.*, 2000). Similarly, taking pravastatin or fluvastatin with or without food did not significantly affect their LDL-C lowering capacities (Pan *et al.*, 1993; Dujovne & Davidson, 1994). In the present study we determined that mean plasma levels of LDL-C and lathosterol were not significantly different in LHSC Lipid Clinic patients presumed to take their daily rosuvastatin dose with or without food. This finding itself is not wholly surprising, as LHSC Lipid Clinic patients have their rosuvastatin doses titrated to effect. However, we also found that the average dose of rosuvastatin was not significantly different between patients presumed to take their rosuvastatin with or without food. These results suggest that taking rosuvastatin with or without food does not significantly affect the amount of rosuvastatin that reaches the liver to inhibit endogenous cholesterol production. Taken together, results from the

present work indicate that taking rosuvastatin with food results in lower rosuvastatin systemic exposure but does not likely affect its therapeutic actions.

A significant impediment to statin therapy is muscle toxicity resulting from high systemic statin exposure (DeGorter *et al.*, 2013). Statin-induced muscle toxicities most commonly present as muscle pain or weakness known as myalgia, occurring in up to 10% of statin patients (Joy & Hegele, 2009). Infrequently, a life-threatening form of muscle toxicity, rhabdomyolysis, may occur as a result of statin therapy (Polderman, 2004). Switching statins to avoid statin-induced muscle toxicities may be efficacious. Indeed, one study reported that 43% of patients that had switched to another statin after an episode of statin-induced myopathy did not experience recurrent symptoms (Hansen *et al.*, 2005). However, it is unclear what effect switching statins had on LDL-C reduction within these patients. Changing a patient's dosing schedule from daily to alternate-day dosing has also been suggested as a means to mitigate statin-induced myalgia (Joy & Hegele, 2009), however statin efficacy may suffer from such an approach. For example, Dulay *et al.* (2009) observed LDL-C reductions of 48.5% in hypercholesterolemic patients taking 10 mg rosuvastatin daily, versus 40.9% when a 20 mg dose of rosuvastatin was given on alternate days. Even small changes in LDL-C reduction can have a profound impact on patient outcome. For example, data from a large meta-analysis by the Cholesterol Treatment Trialists' Collaborators (2005) indicated that a 1 mmol/L reduction in LDL-C that is sustained for 5 years would likely produce a 23% reduction in major vascular events. The present work suggests that taking rosuvastatin with food lowers systemic exposure of rosuvastatin without compromising LDL-C reduction. Therefore, patients may find that taking rosuvastatin with food may prove to be a meaningful strategy for maintaining statin efficacy while mitigating statin-induced muscle adverse effects.

Statins are commonly prescribed to both men and women for the treatment of hyperlipidemia. Although the pharmacokinetic profiles of rosuvastatin are similar in men and women (Martin *et al.*, 2002), studies investigating other statin medications have reported sex-related differences in statin systemic exposure (Gibson *et al.*, 1996; Cheng *et al.*, 1992). In this work, we demonstrated that the effect of food on the oral

pharmacokinetics of rosuvastatin is similar between Caucasian and East Asian men and women. However, female gender has been associated with an approximate 2-fold increase in statin-induced myopathy risk (Feng *et al.*, 2012). Taking rosuvastatin with food may, therefore, prove to be a particularly important strategy for reducing statin-induced myopathy risk within female rosuvastatin patients. Other risk factors for developing statin-induced myopathy where individuals may similarly benefit from a change in rosuvastatin administration behaviour include a history of myopathy, advanced age, high-dose statin therapy, concomitant use of medications known to increase statin systemic exposure, or carriers of select genetic mutations (Joy & Hegele, 2009).

BCRP is an efflux transporter, coded for by *ABCG2*, which plays an important role in the disposition of rosuvastatin (DeGorter *et al.*, 2012c). *ABCG2* c.421C>A has been associated with higher plasma rosuvastatin concentrations in healthy volunteers (Keskitalo *et al.*, 2009b). Moreover, in Chinese patients being treated with 10 mg of rosuvastatin daily, homozygous variant carriers of *ABCG2* c.421C>A showed a 6.9% greater reduction in LDL-C level when compared to homozygous wild-type individuals (Tomlinson *et al.*, 2010). Higher allelic frequencies of *ABCG2* c.421C>A in Asian populations when compared to Caucasian populations may explain the higher plasma rosuvastatin levels previously reported within Asian subjects when compared to Caucasian subjects (Yasuda *et al.*, 2008; Lee *et al.*, 2005). Increased plasma levels of rosuvastatin may predispose Asian patients to severe myopathy. Consequently, the daily maximum approved dose in Asian countries is 20 mg compared with 40 mg within North America (DeGorter *et al.*, 2013). In this study, *ABCG2* c.421C>A allele frequency was much higher in our East Asian cohort when compared to the Caucasian cohort. Furthermore, we found that carriers of *ABCG2* c.421C>A had higher rosuvastatin C_{max} and $AUC_{0-\infty}$ values when compared to non-carriers and that this effect was independent of food administration with rosuvastatin. Taken together, our findings suggest that carriers of *ABCG2* c.421C>A that take their rosuvastatin dose without food may be at an increased risk for high plasma exposure of rosuvastatin and, consequently, developing statin-induced myopathy. Of note, we investigated other SNPs within *ABCG2* and

SLCO1B1 but did not observe any differences with regards to plasma rosuvastatin concentrations between variant carriers and non-carriers.

5.1. Conclusions

In this study we investigated the effect of concomitant food administration on plasma rosuvastatin concentrations in healthy Canadian East Asian and Caucasian volunteers. We also sought to determine whether LHSC lipid clinic patients who took their rosuvastatin dose with food had altered lipid profiles when compared to those that took their dose without food. Finally, we investigated a potential food effect on the plasma and liver levels of rosuvastatin within wild-type C57BL/6 mice. Taken together, these studies provide insight into the effect of food on the oral pharmacokinetics of rosuvastatin.

Conflicting evidence existed with regards to a potential effect of concomitant food administration on plasma rosuvastatin exposure. Through our work in healthy Caucasian and East Asian volunteers and within mice, it is apparent that taking rosuvastatin with food alters its oral pharmacokinetics. Our findings support our previous hypothesis that taking rosuvastatin with food results in lower circulating statin levels. Moreover, the findings from our retrospective analysis of lipid data collected from rosuvastatin patients indicates that taking rosuvastatin with or without food does not affect its LDL-cholesterol lowering capacity.

Statin-induced muscle pain is a major reason for discontinuation of therapy (Abd & Jacobson, 2011). Muscle toxicity is often associated with high statin dose and increased statin plasma exposure (Jacobson, 2006). Here we demonstrate that taking rosuvastatin with food results in lower systemic exposure without compromising its therapeutic benefit. Therefore, our findings have the potential to serve as a novel and simple strategy for mitigating statin myopathy risk. Furthermore, we revealed that carriers of *ABCG2* c.421C>A variant allele have higher plasma levels of rosuvastatin when compared to non-carriers. The risk of excessive rosuvastatin systemic exposure and, consequently, risk of myopathy might be greatest in *ABCG2* c.421C>A variant carriers that consistently take their rosuvastatin dose without food.

Current United States and Canadian dosing guidelines indicate no preference for fed or fasted rosuvastatin administration (Center for Drug Evaluation and Research, 2012). The findings from this work indicate that a modification to these guidelines should be considered, suggesting that rosuvastatin patients take their dose with a meal. Moreover, this modification to the administration guidelines may be particularly important for individuals required to be on high-dose rosuvastatin to meet therapeutic target or for carriers of the *ABCG2* c.421C>A variant allele.

5.2. Limitations

The FDA recognized the potential for food to alter the oral pharmacokinetics of new medications and established guidelines for the design of clinical food-effect studies. The FDA Guidance on Food-Effect Bioavailability and Fed Bioequivalence Studies recommends that medications be administered under fasted and fed conditions, where the fed state consists of a meal around 1000 calories (approximately 50% from fat) in order to maximize the prospects for an observable food effect (Food and Drug Administration, 2002). Neither the low-fat or high-fat meals used within the healthy-volunteer PK study section of this work meet these standards. It is possible that had the test meals been designed to meet the standards proposed by the FDA guidance, a larger food effect could have been demonstrated. However, in designing the test meals for this work, we sought guidance from LHSC dieticians and chose items that are not contraindicated in hyperlipidemic patients. Therefore, we suspect that the magnitude of the rosuvastatin-food effect found within our healthy Caucasian and East Asian cohorts more closely resembles that which would be found within statin patients.

In order to investigate the effect of concomitant food administration with rosuvastatin dose on the lipid profiles of statin patients, we made a number of assumptions regarding the administration behaviours of LHSC lipid clinic patients. First, we used a patient's time since last rosuvastatin dose to infer whether or not they likely took the dose with food. We assumed that individuals who reported taking their last dose between 5-9 am (inclusive) likely did so with breakfast, and those who reported taking their dose between 5-7 pm (inclusive) likely did so with dinner. Furthermore, we presumed that individuals

who reported taking their last dose after 8 pm likely did so before bed and without food. Next, we assumed that patients were likely to routinely take their rosuvastatin dose under similar conditions. Although we recognize that these classifications are unlikely to encompass all individuals, we believe them to be fair estimates as patients (through physician encouragement) tend to link drug doses to aspects of their daily routine such as meal times or before bed (Crammer, 1998). Moreover, the retrospective analysis of LHSC lipid clinic patient data was conducted in a predominantly Caucasian population, and caution should be used when extrapolating these findings to other ethnicities.

We further examined the effect of food on the oral pharmacokinetics of rosuvastatin in wild-type C57BL/6 mice. We used previously collected data from our lab in order to calculate an appropriate sample size of 5 mice per group. The variance in plasma rosuvastatin levels within our mice was larger than expected and, therefore, this work would have benefitted from a larger sample size. Moreover, a potential food effect was assessed by fasting one group of mice for 6 hours prior to drug administration and comparing their plasma levels of rosuvastatin to levels collected from non-fasted mice. Lights were left off during the 6 hour fasting period to promote the normal nocturnal feeding patterns within the mice that had access to food. However, we did not verify if or when these mice consumed the food.

5.3. Future Directions

Both our work and the work done by Li *et al.* (2009), investigate the effect of food on rosuvastatin oral pharmacokinetics in young (average ages between 21-29 years), healthy volunteers. An important follow-up study to the work presented in this thesis would be to investigate the effect of food on rosuvastatin pharmacokinetics prospectively in statin-treated dyslipidemic patients. Specifically, one could design a study whereby rosuvastatin-treated patients are asked to take their dose with a large meal everyday for two weeks and then complete a one-day PK study. The patient could then be asked to switch administration behaviours and begin taking their rosuvastatin dose before bed without food for two weeks and then once again complete a one-day PK study. Alternatively, previous work from our lab has identified 5 hours post dose as the single

time point where blood sampling best correlates with rosuvastatin AUC₀₋₁₀ (DeGorter *et al.*, 2012b), thus a full PK study may not be necessary. Lipid parameters such as LDL-C and lathosterol should be determined from blood samples collected at the end of each two-week period. Rosuvastatin plasma levels and lipid parameters could then be compared between administration methods. Moreover, it may be helpful to survey any changes in muscle fatigue or discomfort throughout the study period. Care should be taken when implementing such a study design in patients on a high dose of rosuvastatin or *ABCG2* c.421C>A variant-allele carriers.

In this work, we found that administering an oral dose of rosuvastatin to fed mice resulted in lower plasma statin concentrations when compared to values obtained within fasted mice. Moreover, the mean liver-to-plasma concentration ratio was approximately 2.1-fold greater in fed mice when compared to fasted mice, but this difference was not significant. Detecting a significantly higher mean liver-to-plasma concentration ratio within the fed mice would provide support for the hypothesis that administering rosuvastatin with food results in enhanced hepatic disposition. A future study with a larger sample size may provide better insight into a potential mechanism for the observed food effect. Furthermore, dogs have been identified as the most appropriate animal model for understanding or predicting the effect of food on drug pharmacokinetics within humans, and a study investigating the effects of food on rosuvastatin pharmacokinetics in dogs might further aid in elucidating a food-effect mechanism (Lentz, 2008).

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Appendices

Appendix A: Ethics Approvals for Human Pharmacokinetic Study



**Western
Research**

Use of Human Participants - Ethics Approval Notice

Research Ethics

Principal Investigator:Dr. Richard Kim
File Number:103870
Review Level:Full Board
Approved Local Adult Participants:25
Approved Local Minor Participants:0
Protocol Title:Evaluation of food effects on the oral pharmacokinetics of rosuvastatin
Department & Institution:Schulich School of Medicine and Dentistry\Medicine-Dept of,London Health Sciences Centre
Sponsor:
Ethics Approval Date:June 10, 2013
Ethics Expiry Date:June 01, 2014

Documents Reviewed & Approved & Documents Received for Information:

Document Name	Comments	Version Date
	Telephone correspondence	2013/05/08
Advertisement	Advertisement	
	Initial Assessment Form	2013/05/24
	Data Collection Form	
Letter of Information & Consent		2013/05/24


This is to notify you that the University of Western Ontario Health Sciences Research Ethics Board (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this HSREB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the University of Western Ontario Updated Approval Request form.

Member of the HSREB that are named as investigators in research studies, or declare a conflict of interest, do not participate in discussions related to, nor vote on, such studies when they are presented to the HSREB.

The Chair of the HSREB is Dr. Joseph Gilbert. The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Ethics Officer to Contact for Further Information

 Erika Basile	Grace Kelly	Shantel Walcott
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Use of Human Participants - Revision Ethics Approval Notice

Principal Investigator: Dr. Richard Kim
 File Number: 103870
 Review Level: Delegated
 Protocol Title: Evaluation of food effects on the oral pharmacokinetics of rosuvastatin
 Department & Institution: Schulich School of Medicine and Dentistry/Medicine-Dept of, London Health Sciences Centre
 Sponsor:
 Ethics Approval Date: June 10, 2013 Expiry Date: April 30, 2015
 Documents Reviewed & Approved & Documents Received for Information:

Document Name	Comments	Version Date
Revised Study End Date	study end date extension to June 30, 2015	

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced revision(s) or amendment(s) on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the University of Western Ontario Updated Approval Request Form.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the HSREB.

The Chair of the HSREB is Dr. Joseph Gilbert. The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Ethics Officer to Contact for Further Information

<input checked="" type="checkbox"/> Erika Basile	<input type="checkbox"/> Grace Kelly	<input type="checkbox"/> Mina Mckhail	<input type="checkbox"/> Vikki Tran
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This is an official document. Please retain the original in your files.

Appendix B: Ethics Approval for Mouse Pharmacokinetic Study



AUP Number: 2015-072

PI Name: Kim, Richard

AUP Title: Rosuvastatin Distribution In The Fasted And Fed State

Approval Date: 02/16/2016

Official Notice of Animal Use Subcommittee (AUS) Approval: Your new Animal Use Protocol (AUP) entitled "Rosuvastatin Distribution In The Fasted And Fed State" has been APPROVED by the Animal Use Subcommittee of the University Council on Animal Care. This approval, although valid for four years, and is subject to annual Protocol Renewal.2015-072::1

1. This AUP number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this AUP number.
3. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

Submitted by: Copeman, Laura
on behalf of the Animal Use Subcommittee
University Council on Animal Care

Curriculum Vitae

Name: Cheynne McLean

Post-secondary Education and Degrees: Western University, London ON
 Bachelor of Medical Sciences, Honors Specialization
 2010-2014

Western University, London ON
 MSc Candidate, Physiology & Pharmacology
 Sept. 2014 - Present

Honours and Awards: Western Continuing Scholarship of Distinction
 Sept. 2010

Western University: Dean's Honour Roll
 May 2011-2014

Western In-Course Scholarships Year III & IV
 May 2012 & 2013

Frederick Banting and Charles Best Canada Graduate Scholarship-
 Master's (CGS M)
 April 2015

Ontario Graduate Scholarship (OGS)
 May 2015
 Offer Declined

Related Work Experience: Research Assistant: Department of Medicine
 London Health Sciences Centre, ON
 May 2014 - Sept. 2014

Teacher's Assistant: Department of Pharmacology
 Western University, ON
 Human Pharmacology and Therapeutic Principles
 Sept. 2014 - April 2016

Publications:

McLean, C., Morse, B., Gryn, S., & Kim, R. B. (2015, April). *Evaluation of Food Effects on the Oral Pharmacokinetics of Rosuvastatin*. Poster presented at the annual London Health Sciences Research Day, London, ON.

McLean, C., Morse, B., Gryn, S., & Kim, R. B. (2015, June). *Food for Thought: The Impact of Dietary Fats on the Oral Pharmacokinetic Profiles of Rosuvastatin*. Poster presented at the annual meeting of the Canadian Society of Pharmacology and Therapeutics, Toronto, ON.

Russell, L., Gulilat, M., Schwarz, U., Teft, W., **McLean, C.**, & Kim, R. B. (2016, March). *Identifying Clinically Important Genetic Variants in Bile Acid Transporters Using Next-Generation Sequencing*. Poster presented at the annual London Health Sciences Research Day, London, ON.

Wilson, A., **McLean, C.**, & Kim, R. B. (2016). Trimethylamine-N-oxide: a link between the gut microbiome, bile acid metabolism, and atherosclerosis. *Current opinion in lipidology*, 27(2), 148-154.

McLean, C., Wilson, A., & Kim, R. B. (2016). Impact of Transporter Polymorphisms on Drug Development: Is it clinically significant? *Journal of Clinical Pharmacology*. In press.