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Computational Modelling of Simvastatin - Effects on HMG-CoA Reductase Activity and Cholesterol Levels

vorgelegt von **Florian Bartsch**
Matrikelnummer: 588830
Geburtsdatum/-ort: 26.09.1997, Berlin
Email-Adresse: bartsflo@hu-berlin.de

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Betreuer und Erstprüfer: **Dr. Matthias König**

Zweitprüfer: **Prof. Dr. Hanspeter Herzel**

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Abstract

English

Cholesterol is one of the most important molecules in biology. Elevated plasma levels are associated with an increased risk for arteriosclerosis and cardiovascular diseases. A common treatment for hypercholesterolemic patients are statins, which are capable of reducing total plasma cholesterol levels. Within this thesis the effects of the statin simvastatin on cholesterol levels were studied using a computational modelling approach. Based on extensive data curation, a kinetic model of simvastatin pharmacokinetics coupled to a pharmacodynamic model of cholesterol metabolism was developed. The resulting pharmacokinetic/ pharmacodynamic (PK/PD) model was applied to study the effects of simvastatin on hepatic HMG-CoA reductase activity, hepatic cholesterol homeostasis and plasma cholesterol levels.

German

Cholesterin gehört zu den wichtisten Molekülen in der Biologie. Erhöhte Konzentrationen im Blut sind mit einem erhöhten Risiko für Arteriosklerose und Herz-Kreislauf-Erkrankungen verbunden. Häufig verwendete Medikamente zur Behandlung von Hypercholesterinämie, die zu einer Senkung des Cholesterinspiegels führen können. Im Rahmen dieser Arbeit wurde die Wirkung des Statins Simvastatin mit Hilfe eines computergestützten Stoffwechselmodells untersucht. Nach umfangreicher Datenkuration wurden Daten zur Pharmakokinetik und -dynamik von Simvastatin und Cholesterol zusammengestellt. Auf dieser Grundlage wurde ein pharmakokinetisches Modell (PK) von Simvastatin, gekoppelt an ein pharmakodynamisches Modell (PD) des Cholesterinstoffwechsels, entwickelt. Das resultierende Modell (PK/PD) wurde benutzt, um die Auswirkungen von Simvastatin auf die hepatische Aktivität der HMG-CoA Reduktase, die hepatische Cholesterinhomöostase und die Cholesterinspiegel im Blut zu untersuchen.

1 Introduction

Cholesterol is one of the most important and most highly decorated molecules in biology. Overall, thirteen Nobel Prizes have been awarded for research on this molecule [5]. Cholesterol is part of every animal cell covering many functions. Due to its importance, abnormalities of the cholesterol metabolism and levels can result in serious diseases. A common disorder are elevated plasma levels of total cholesterol (hypercholesterolemia) which are associated with an increased risk for arteriosclerosis and cardiovascular diseases [60]. A standard treatment for hypercholesterolemia are statins, drugs capable of decreasing plasma cholesterol levels.

Within this thesis a kinetic model of simvastatin pharmacokinetics (PK) coupled to a pharmacodynamic (PD) model of cholesterol metabolism was developed. The resulting PK/PD model was applied to study the effects of simvastatin on hepatic HMG-Coenzyme A reductase activity, hepatic cholesterol turnover and plasma cholesterol levels.

1.1 Cholesterol

Cholesterol (Fig. 1) as one of the most important molecules in biology covers a variety of essential functions in animal cells. It acts as a structural component in cell membranes, interacting with lipids and numerous transmembrane proteins to regulate fluidity, rigidity, and permeability of cellmembranes [60]. In addition, cholesterol acts as a precursor for many biomolecules such as bile acids, vitamin D and steroids [14].

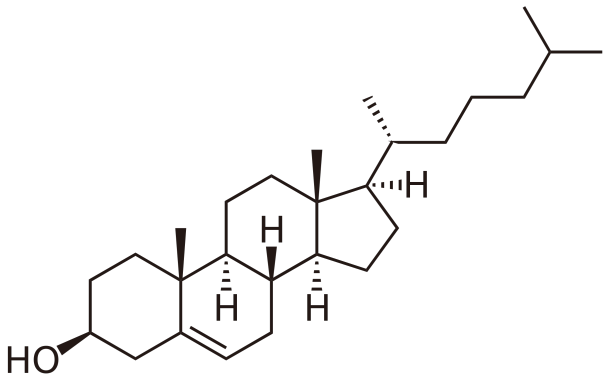


Figure 1: Chemical structure of cholesterol. (CHEBI:16113, inchikey: HVYWMOMLDIMFJA-DPAQBDIFSA-N) (chemical structure from ChEBI [28])

Multiple routes and pathways are involved in cellular cholesterol homeostasis in the human body. The most important being dietary uptake, cholesterol synthesis, cholesterol utilization and excretion. The balance and the contributing routes vary a lot in the population.

It is assumed that the gut absorbs a maximum of 1 g of dietary lipids a day [4]. Dietary cholesterol is taken up by the intestine and distributed in the body via lipoproteins (sec. 1.1.1).

De novo cholesterol biosynthesis contributes 0.6 to 1.2 g/day [12, 4] to the cholesterol pool.

Being part of the systemic circulation, cholesterol utilization can take place in all parts of the body. Its excretion is mainly into the feces as unchanged cholesterol or as bile acids. Whole body utilization and fecal loss are reported to contribute approximately 1.6 g/day [12].

1.1.1 Lipoproteins

Cholesterol as a lipid can be hardly solubilized in water and is too hydrophobic to cross biomembranes passively. To transport cholesterol with the blood, it is packed in lipoproteins mediated by lipoprotein receptors (Fig. 2) [5]. Lipoproteins are particles with a surface of a phospholipid monolayer, with integrated free cholesterol and lipoprotein-specific apolipoproteins. The core contains cholesterol esters and triglycerides [18].

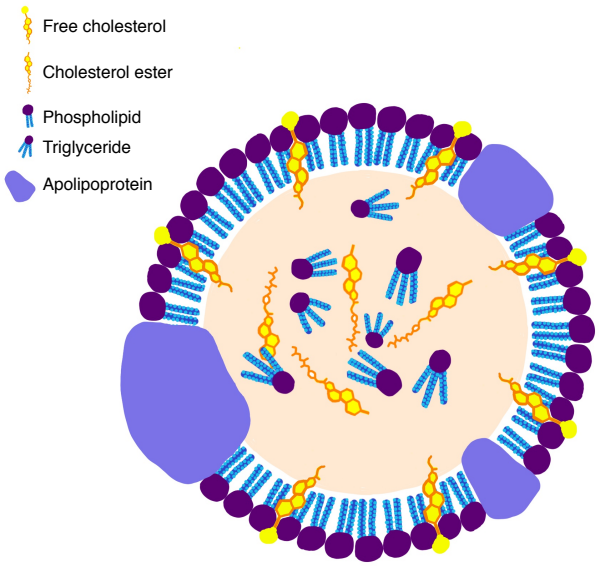


Figure 2: Lipoprotein structure. Lipoproteins consist of a phospholipid monolayer with integrated free cholesterol molecules and lipoprotein specific apolipoproteins. Inside of the monolayer, the lipoprotein contains cholesterol esters and triglycerides.

Apolipoproteins are not only important for the structure of lipoproteins,

but act as ligands for lipoprotein receptors. They are involved as regulators for lipoprotein-metabolism [18].

Lipoprotein particles are differentiated based on their lipid composition, size, density and type of apolipoproteins. Important lipoproteins are HDL (high-density-lipoproteins), IDL (intermediate-density-lipoprotein), VLDL (very-low-density-lipoproteins), LDL (low-density-lipoprotein), and chylomicrons (also known as ULDL, ultra low-density lipoproteins).

An overview on how these particles are involved in the transport of cholesterol is given in Fig. 3.

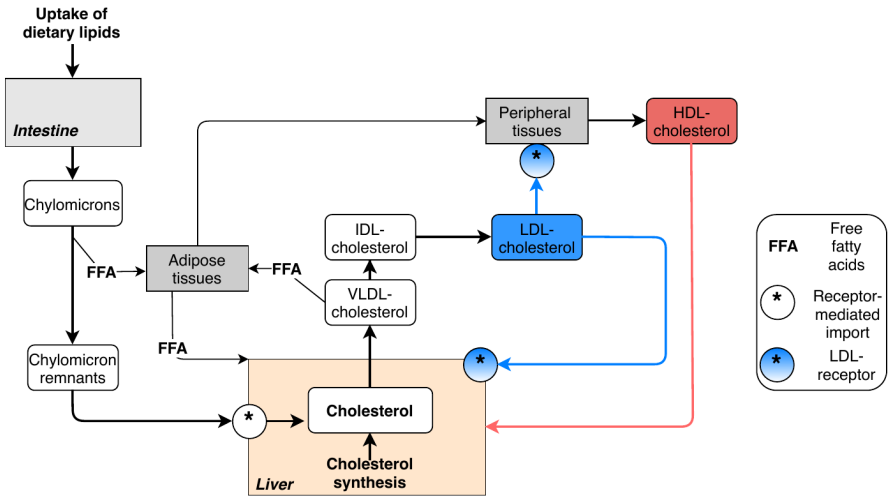


Figure 3: Uptake and distribution of lipids and cholesterol via lipoproteins. Dietary lipids are absorbed by the intestine and stored in chylomicrones (ULDL). Free fatty acids in chylomicrones can be taken up by peripheral and adipose tissue cells. Via the release of free fatty acids the chylomicrones are converted to chylomicrone remnants, which, with their cholesterol and remaining lipids, are taken up by the liver through receptor mediated endocytosis. The liver is the main site of cholesterol biosynthesis. Synthesized cholesterol is packed into VLDL particles and excreted into the bloodstream. VLDL releases free fatty acids to adipose tissue and is converted into IDL and subsequently into LDL. LDL transports cholesterol and other lipids to peripheral tissues or can be taken up by the liver through LDL-receptor mediated endocytosis. Cholesterol from peripheral tissues is transported back to the liver by HDL particles.

Dietary cholesterol is taken up by the intestine and released in the circulation with other dietary lipids in form of chylomicrones (ULDL). By releasing free fatty acids they are converted to chylomicrone remnants, which with their cholesterol and remaining lipid content are taken up by the liver through receptor mediated endocytosis [18].

The liver is the main site of cholesterol biosynthesis. Hepatic cholesterol is secreted as part of VLDL particles to the circulation, where VLDL is processed via IDL to LDL [60]. VLDL-cholesterol particles are high in triglycerides. By releasing free fatty acids from the VLDL particles to the adipose

tissue they are converted to IDL, which are converted after further lipid release to LDL [18]. LDL particles are richer in cholesterol than VLDL and IDL and are the main involved lipoproteins in the transportation of cholesterol from the site of synthesis to peripheral tissues. LDL can be taken up by the liver through LDL-receptor-mediated endocytosis thereby increasing cellular cholesterol levels. Each lipoprotein has lipoprotein-specific apoproteins, which mediate transport and endocytosis [80]. HDL transports cholesterol from peripheral tissues back to the liver [18].

1.1.2 Cholesterol biosynthesis

Cholesterol biosynthesis (Fig. 4) is mainly located in the liver which accounts for 50-70% of the total synthesis capacity [9, 60]. It is a complex pathway involving more than 20 enzymes located in the endoplasmatic reticulum and cytoplasm [60].

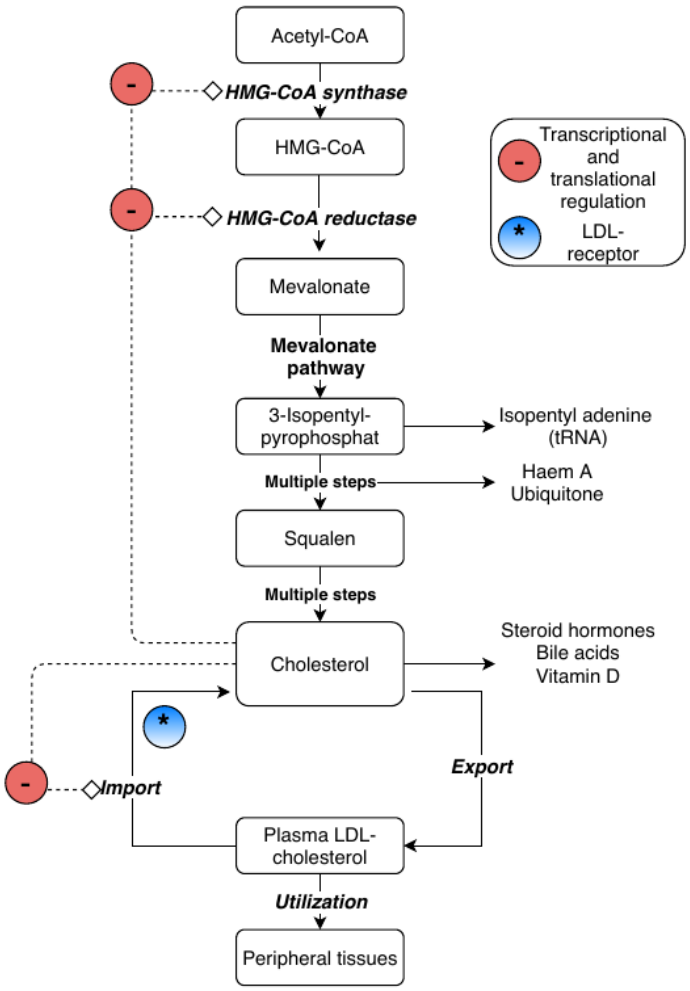


Figure 4: Schematic overview of cholesterol biosynthesis. Cholesterol is synthesized from the precursor acetyl-CoA in a series of reaction steps. Key initial enzymes of the cholesterol biosynthesis are HMG-CoA synthase and HMG-CoA reductase, both negatively regulated by hepatic cholesterol levels via transcriptional and translational mechanisms. Intermediates of the cholesterol synthesis pathway are important precursors, for instance for ubiquitone, steroid hormones or vitamin D. Cholesterol from LDL can be imported via LDL receptor mediated uptake or can be exported as VLDL. LDL-receptor expression is negatively regulated via hepatic cholesterol levels.

Acetyl-Coenzyme A (acetyl-CoA) is the main cholesterol precursor, with all of the 27 carbon atoms of cholesterol originating from acetyl-CoA molecules [14]. Cholesterol synthesis includes three main reaction steps, introduced in the following section (see: https://reactome.org/content/detail/R-HSA-191273 for overview).

The initial step is the formation of acetoacetyl-CoA from two molecules acetyl-CoA. Then, the enzyme HMG-CoA synthase hydrolyzes acetoacetyl-CoA

with an additional molecule acetyl-CoA to 3-Hydroxy-3-methylglutaryl-Coenzyme A (HMG-CoA).

The next reaction is the irreversible reduction of HMG-CoA to mevalonate mediated by the HMG-CoA reductase in the endoplasmatic reticulum. This step is the main rate limiting step in cholesterol biosynthesis. Regulation of this step, through regulation of this enzyme is important to maintain hepatic cholesterol levels. Three ATP-dependent reactions catalyze the formation of 3-isopentylpyrophosphat from mevalonate via the mevalonate pathway.

Six molecules 3-isopentylpyrophosphat form squalen in the endoplasmatic reticulum, which is catalyzed in the third main step via the intermediates 2,3-epoxysqualene, lanosterol and other metabolites to the end product cholesterol [76].

1.1.3 Regulation of cholesterol biosynthesis

The biosynthesis of cholesterol is an energetically expensive process. In its multiple steps, it requires acetyl-CoA, ATP, oxygen, NADPH and NADH and involves several key enzymes. Due to these energetic costs and the importance of cholesterol and its precursors, the biosynthesis of cholesterol is tightly regulated. The regulatory mechanisms ensure constant cellular cholesterol and mevalonate levels as well as avoiding sterol over-accumulation[24, 60].

A key regulatory mechanism is the modulation of the amount of key enzymes via feedback regulation on transcriptional and translational level. Either cholesterol directly or other intermediates of the cholesterol biosynthesis are involved in this modulation.

LDL-cholesterol and cellular cholesterol levels can be sensed by the cell. As LDL-cholesterol in the plasma is derived from cholesterol, it is an indicator for cellular cholesterol levels. When LDL-cholesterol levels are low, the activities of HMG-CoA synthase and HMG-CoA reductase are held high, in order to maintain mevalonate and cholesterol production. Studies in human fibroblasts examined the effects on the HMG-CoA reductase after the withdrawal of lipoproteins. They found that the activity of HMG-CoA reductase was 50-fold increased after the removal of lipoproteins in a cultured medium. When lipoproteins were added back, the enzyme activity rose again [5].

Sterol regulatory element-binding proteins (SREBP) play an important role in the transcriptional regulation. SREBPs are transcriptional regulators of proteins involved in fatty acid and triglyceride synthesis (SREBP1) and cholesterol biosynthesis (SREBP2) [31].

Low hepatic cholesterol levels are sensed by the cell, resulting in an increased nuclear translocalization of SREBP2, followed by transcription of target genes. This leads to increased expression rates of proteins involved in cholesterol biosynthesis such as the HMG-CoA reductase, HMG-CoA synthase and the LDL receptor. The LDL-receptor mediates the uptake of cholesterol from plasma LDL-cholesterol particles. The increased receptor and HMG-CoA reductase and synthase expression lead to an enhanced uptake of cholesterol into the cell and enhanced biosynthesis of cholesterol. Sterol repletion results in a deactivation of transcription and activation of proteasomal degradation of SREBP2, resulting in decreased synthesis and LDL-cholesterol uptake rates [60].

1.1.4 Hypercholesterolemia

Hypercholesterolemia is a lipid disorder characterized by elevated plasma LDL- and total cholesterol levels. Hypercholesterolemia is associated with an increased risk for atherosclerosis, which can lead to cardio-, cerebro- and peripheral morbidity and mortality [9]. Cholesterol levels became an important indicator for increased risks for cardiovascular diseases (CVD), which are often followed by death [1]. Globally CVD lead to approximately 17.8 million deaths in 2017, increasing by 21.2% in comparison to 2007 [92]. Especially patients with additional risk factor such as Type 1 or 2 diabetes, smoking, low physical activity or high blood pressure are endangered for CVD by elevated blood lipid levels [30].

Based on European guidelines from 2003 total plasma cholesterol and LDL-cholesterol should not be higher than 5 mmol/l and 3 mmol/l, respectively. The cut off values for high risk patients are reported as 4.5 mmol/l and 2.5 mmol/l for total- and LDL-cholesterol. High risk patient are patients with at least one or the combination of several risk factors [1].

Main reasons for elevated blood lipids are lifestyle factors or genetic disorders (familiar hypercholesterolemia). Lifestyle factor are mostly dietary habits, such as increased intakes of saturated and trans fatty-acids [35].

Familiar hypercholesterolemia (FH) is developed due to genetic mutations in the gene encoding the LDL-receptor. This results in decreased expression rates or loss of function for the receptor [35]. Consequences are delayed uptake and clearance rates of plasma LDL-cholesterol, resulting in elevated plasma levels. FH can be differentiated in homozygote and heterozygote. Patients with homozygote FH have two mutated LDL receptor alleles, leading to higher LDL-cholesterol levels, than in patients with heterozygote FH, who have only a single mutated allele [35].

1.2 Statins

Hypercholesteremia is typically treated with dietary changes and/or medication. Most used drugs are statins, which are capable of inhibiting the HMG-CoA reductase (Fig. 4), resulting in a reduction of plasma LDL-cholesterol and total cholesterol [56].

Statin usage increased massively in the last 20 years. A study reported an increase of 7.2% per year from 2000 to 2012 as the mean from several western European countries. The DDD (daily defined dosis) of simvastatin per thousand inhabitants per day increased from 22 in 2000 to 95 in 2012 [89].

There are several statins available on the market including lovastatin, pravastatin, fluvastatin, atorvastatin, rosuvastatin and simvastatin, which is the most frequently used statin. A study from Denmark reported an increased usage of simvastatin from 55% in 1996 to 74% of total statin usage in 2012. Until 2015 the simvastatin usage as a fraction of total statin usage decreased to 53% [64].

From a chemical perspective statins can be differentiated based on their synthetic background and functional groups, varying in their hydro- and lipophicility [36]. Type-1 statins (lovastatin, pravastatin and simvastatin) reuse the structure of mevastatin, which is derived from the fungus *Aspergillus terreus*. These statins are either non-synthetic (lovastatin) or semi-synthetic (simvastatin, pravastatin) and are administered as prodrugs. This means that their ad-

ministered form has no activity and needs to metabolized to their active form. Type-2 statins are fully synthetic with larger functional groups (Fluvastatin, Atorvastatin, Rosuvastatin) and are administered as active compounds [9].

1.2.1 Simvastatin metabolism

As simvastatin is the main focus of this work, its metabolism is introduced in this section. Simvastatin undergoes extensive first-pass-metabolism in the intestine and liver [38]. Most of the drug is metabolised before reaching the circulation. The metabolism of simvastatin is catalyzed by cytochrome P450 (CYP), by esterases and to some extent by non-enzymatically catalyzed reactions [55]. CYP-enzymes are the main enzymes involved in the first pass effect, accounting for 75% of drug metabolism, while esterases are believed to contribute 10% [20].

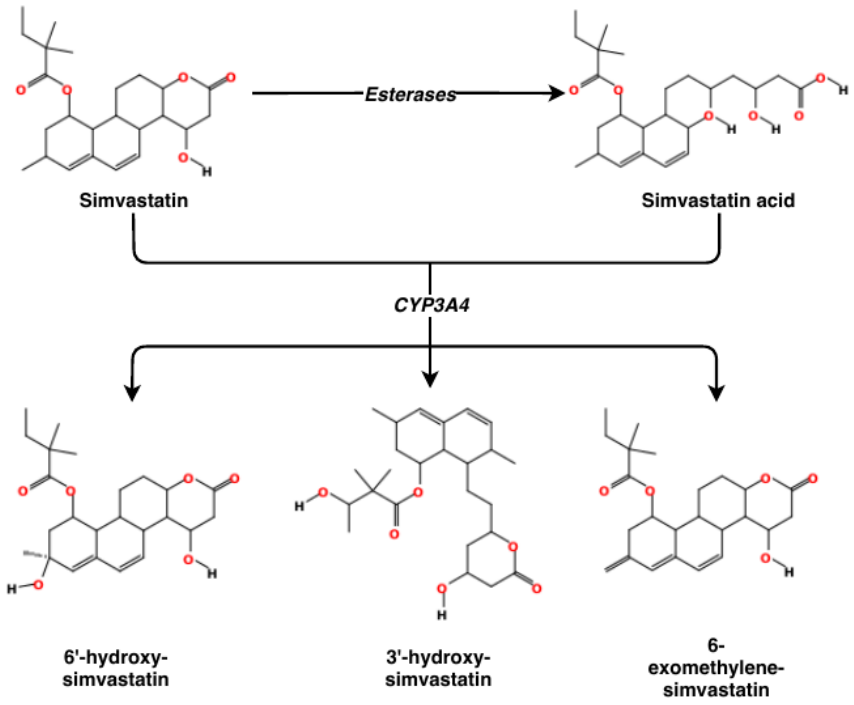


Figure 5: Two classes of enzymes play a role in the metabolism of simvastatin. Esterases convert simvastatin to simvastatin acid. Both, simvastatin and simvastatin acid can be converted to several metabolites by CYP3A4. Depicted are some of the main metabolites after CYP3A4-hydrolysis (6'-hydroxy-simvastatin, 3'-hydroxy-simvastatin and 6-exomethylene-simvastatin). Esterases are mainly located in the liver and plasma, while CYP3A4 is mainly located in the liver and small intestinal wall.

CYPs are oxygenases that catalyze hydroxylation-reactions [27]. Their main site of action are the liver and small intestinal wall [55]. CYPs play a major role in the metabolism of drugs and other xenobiotics. The metabolism of

simvastatin and simvastatin acid is mainly mediated by CYP3A4 and CYP3A5 (together more than 80%), with CYP3A4 being the dominating isoform. Other CYP isoforms such as CYP2C8 play a minor role (less than 20%) [78].

Esterases are a class of enzymes, which are mainly involved in the activation of prodrugs. They can be differentiated based on substrate specificity and sensitivity towards inhibitors. Main esterases involved in drug metabolism are paraoxonases (PON) and carboxylesterases (CES). PONs belong to A-esterases and hydrolyze aromatic esters. They are mainly located in the liver and plasma. CESs belong to the B-esterases and are members of the serine esterase superfamily. They are mainly involved in the activation of prodrugs in the liver, lung, small intestine and kidney. C-esterases are not believed to be involved in major drug metabolism [20].

The activation of the inactive lactone simvastatin to its main active metabolite simvastatin acid is mainly driven by esterases and non-enzymatically catalyzed reactions, which reversibly convert simvastatin to simvastatin acid in the liver and to some extent in the plasma [55].

CYP3A4-enzymes do not play a major role in the activation of simvastatin, but hydrolyze simvastatin and simvastatin acid to multiple other metabolites. Some of these metabolites can have inhibitory activity, but none of them are as active as simvastatin acid. Important metabolites with a notable inhibitory activity, are simvastatin 6'-carboxylic acid and 6'-hydroxy-simvastatin and 6'-hydroxymethyl-simvastatin, with a activity of 40%, 50% and 90% of the activity of simvastatin acid, respectively [91, 53].

1.2.2 Simvastatin pharmacokinetics

Pharmacokinetics characterize the kinetics and behaviour of a drug after administration. It describes the absorption, distribution, metabolism and elimination (ADME) processes quantitatively and overviews how the body handles a substance.

The metabolic conversion of simvastatin has been introduced in the previous section 1.2.1. Within this section an overview over absorption, distribution, elimination and pharmacokinetic properties of simvastatin and its metabolites is provided.

Absorption Typically simvastatin is applied orally, leading to rapid absorption by the gut and liver. It appears in the plasma circulation with a time to peak concentration t_{max} of 1.3 - 2.4 hr. The C_{max} of simvastatin is dose-dependent and varies in a range of 10 - 34 ng/ml [15].

The gut plays an important role in the absorption of orally given substances because of the effect that some fraction of a drug is directly excreted into the feces without appearing in the gut. For simvastatin 60 - 80% of a dose are absorbed by the gut, while the rest is excreted into the feces [45].

Distribution Distribution kinetics mainly depend on chemical properties of molecules with the polarity playing a major role. Simvastatin as a lactone is a highly lipophilic, non-polar compound. It passes biomembranes passively via diffusion. After being converted by esterases and CYP3A4 enzymes, simvastatin acid and other metabolites gain polarity and hydrophilicity and can not cross biomembranes passively. They are subject to active membrane transporters.

Hepatic simvastatin acid influx is mediated by the OATP1B1 transporter [38]. The role of transporters for the hepatic efflux of simvastatin and simvastatin acid is discussed. Initially, PgP-transporters (P-glycoprotein-transporters) were believed to mediate the efflux. Studies examined the different genes underlying PgP- and OAT1B1-transporters and reported that only genetic variations for the OAT1B1-transporter resulted in significant changes in simvastatin acid pharmacokinetics [38]. Additionally studies examined the mediated efflux of PgP-transporters for simvastatin and simvastatin acid and concluded that simvastatin and simvastatin acid are not significantly subjects to PgP-transporters [29].

Studies in rats report accumulation of simvastatin acid and metabolites in the liver, supporting the findings which negate the role of PgP-transportes. This establishes a tissue specificity for simvastatin and metabolites in the liver. This effects is beneficial because it allows the inhibitory activity to unfold its whole potential in the liver where the cholesterol biosynthesis is mainly located [22].

Hepatic simvastatin and metabolites are subjects to enterohepatic circulation. They are imported into the bile and are transported with the bile flow back into the gut. There, simvastatin and metabolites can either enter systemic circulation again or can be excreted into the feces [52]. Studies in dogs examined the fraction of simvastatin and simvastatin acid, that was excreted in the bile after an intravenous infusion of radiolabbeled simvastatin or simvastatin acid. After intravenous infusion of simvastatin the recovered dose of simvastatin and simvastatin acid were 1.6 and 3.5 % and 4.1 and 1 % respectively. After infusion of simvastatin acid the recovered doses for simvastatin and simvastatin acid were 11.0 and 13.8 % and 13.3 and 13.2 % respectively [90].

Simvastatin and simvastatin acid are highly plasma bound (98 and 95 %) and the bioavailabiliy of simvastatin is only 5 % [65]. The area under the curve extrapolated until infinity $AUC_{0-\infty}$ after an 60 mg dose of simvastatin were 46.6 $\pm$ 5.8 and 21.7 $\pm$ 4.5 ng/ml·hr for simvastatin and simvastatin acid. The $AUC_{0-\infty}$ after the same dose for active and total simvastatin inhibitors were reported as 88 $\pm$ 6.9 and 243 $\pm$ 15.4 ng/ml·hr [53].

The volume of distribution after oral doses of different brands of capsules with 20 mg simvastatin were reported as 19.255 $\pm$ 13.658 and 16.222 $\pm$ 8.969 litre for simvastatin and 18.117 $\pm$ 15.193 and 27.324 $\pm$ 20.524 litre for simvastatin acid [86].

Elimination Simvastatin is mainly eliminated in the feces after hepatic biliary excretion. Studies report that 12.9 $\pm$ 2.4 % total radioactivity of orally administered radiolabelled simvastatin was found in urine and 57.6 $\pm$ 9.4 % in feces [17]. Manufacturer information report that little or no unchanged simvastatin or simvastatin acid can be found in the feces and urine after intravenous infusion. After infusion of simvastatin acid only 0.3 % were found in the urine as inhibitory active compounds [23].

The excretion pharmacokinetic parameters half-life t_{half} and clearance cl of simvastatin are 2 - 5 hours [6] and 0.45 l/hr/kg [15], respectively. Studies report elimination constants k_{el} after administration of two different brands of simvastatin (40 mg oral tablets) of 0.22 and 0.28 1/hr for simvastatin and 0.15 and 0.18 1/hr for simvastatin acid [69].

1.2.3 Simvastatin pharmacodynamics

Simvastatin is used as a treatment for elevated plasma cholesterol levels in hypercholesterolemic patients. Typical doses of simvastatin are 5 to 80 mg daily. The recommended dose of treatment to start a therapy is in the range of 10 to 40 mg simvastatin per day. A dose of 80 mg is very unlikely and is just taken by patients with severe hypercholesterolemia at a high risk for cardiovascular diseases [23]. This sections provides the insights into the effects of simvastatin on cellular and plasma cholesterol levels.

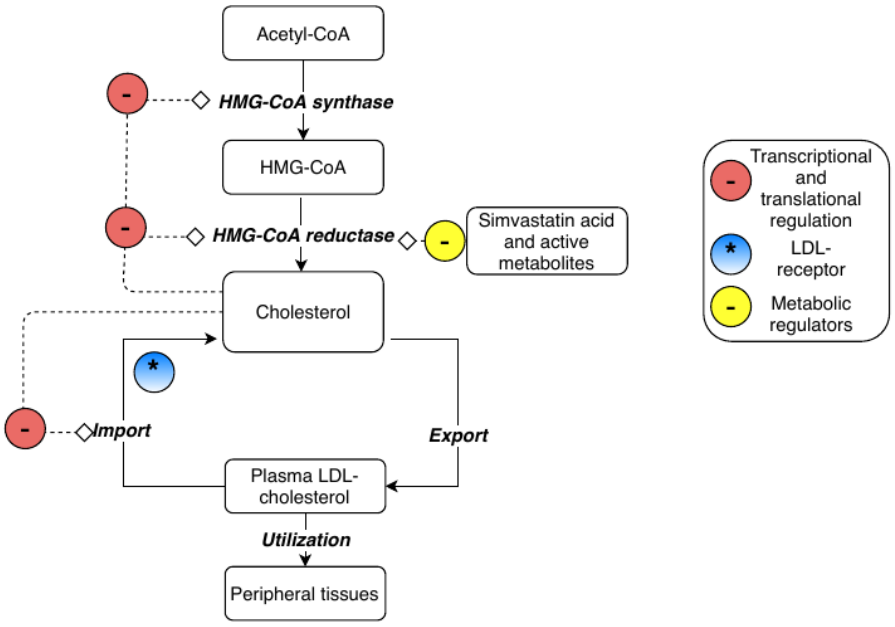


Figure 6: Inhibition of cholesterol synthesis by simvastatin acid. Main steps in cholesterol biosynthesis analogue to Fig. 4, including effects of simvastatin acid and active simvastatin metabolites. The reaction chain is shortenend, starting with the major cholesterol precursor acetyl-CoA and presents just one intermediate (HMG-CoA). The enzymes HMG-CoA synthase and reductase are shown too, as well as the LDL-receptor. The negative feedback inhibition for the expression of these proteins through cellular cholesterol levels are included. This figure includes the inhibition of simvastatin acid and other active metabolites on the enzyme HMG-CoA reductase, which results in decreased activity of this key enzyme.

The chemical form of simvastatin acid and other active simvastatin metabolites are structurally related to the molecule HMG-CoA, which is the main substrate for the HMG-CoA reductase. The structure analogy gives simvastatin acid and other active metabolites the capability to bind to this enzyme, making them competitive inhibitors for the HMG-CoA reductase. The whole inhibitory potential is calculated by the sum of simvastatin acid and other active metabolites with the respective inhibitory activity. [16]. Simvastatin acid accounts for

25 % of this total inhibitory activity [3]. The sum of simvastatin acid as the main active metabolites and other active metabolites are called active HMG-CoA reductase inhibitors. They are differentiated to total HMG-CoA reductase inhibitors, which are the sum of the active inhibitors and metabolites that are not activated yet, but can have inhibitory potential after further metabolization. The difference is quantified by the latent inhibitory activity, describing the time after which other metabolites are activated [75].

The competitive inhibition of active HMG-CoA reductase inhibitors as the rate-limiting step in cholesterol biosynthesis, leads to short and long-term effects. Short term effects are the reduced synthesis of mevalonate, decreasing the flux through cholesterol synthesis, reducing hepatic cholesterol levels. This leads to long-term transcriptional adaptive reactions in the cholesterol homeostasis system. The decrease of hepatic cholesterol leads to an enhanced expression of LDL-receptors (to increase LDL-cholesterol uptake) and HMG-CoA reductase and synthase protein (to increase flux through the mevalonate pathway and cholesterol synthesis). The uptake of LDL-cholesterol and LDL-cholesterol-precursors (VLDL, IDL) from the blood is triggered [61].

Studies report an 200-fold concentration increase after a few hours in HMG-CoA reductase proteins due to adaptive reactions on transcriptional levels. This increase is a multiplicative effect starting with induced transcription on mRNA, followed by higher rates of translation of mRNA, leading to higher enzyme amounts. But even though more HMG-CoA reductase and synthase enzymes are expressed, the HMG-CoA reductase is still strongly inhibited by simvastatin acid and active metabolites, making the over-expression of these enzymes ineffective in renormalizing hepatic cholesterol levels [24].

The other control mechanism is the the increased expression of LDL receptors, which mediate an enhanced uptake of LDL and LDL-precursors from the circulation. Hepatic cholesterol levels increase and plasma LDL-cholesterol levels decrease, making simvastatin an effective plasma cholesterol lowering medication [61].

In summary, not the inhibition of the HMG-CoA reductase and cholesterol biosynthesis is the main mechanism for plasma LDL-cholesterol reduction, but the increased uptake of LDL-cholesterol via up-regulated LDL-receptors.

1.3 Pharmacokinetic/pharmacodynamic model (PK/PD)

Physiological-based pharmacokinetic/ pharmacodynamic models allow to examine the pharmacokinetics and pharmacodynamics of drugs in the body computationally. This allows to elucidate mechanisms and to understand complex biological systems better, such in this work the effects of simvastatin on HMG-CoA reductase activity and plasma cholesterol levels.

Pharmacokinetic parameters can be predicted by a such a mathematical model based on absorption, distribution, metabolism and elimination (ADME). Representing body tissues, those models consist of compartments, which are connected by flow rates, accounting for blood circulation. These tissue compartments include tissue-specific processes describing ADME and are modelled as transport or metabolic reactions. An example would be the metabolic conversion of simvastatin to simvastatin acid in the liver. Physiological-based pharmacokinetic model are able to predict the pharmacokinetics of modelled drugs and their metabolites [39].

A pharmacodynamic model describes the biological or physiological effect of a substance on the body. In this thesis the pharmacodynamic effect of simvastatin and its metabolites on cholesterol biosynthesis, homeostasis and plasma levels were studied.

1.4 Question, scope and hypothesis

Understanding how simvastatin affects cholesterol levels is important. Many older people take multiple medications (among them a statin) resulting in drug-drug interactions with simvastatin. Better understanding these effects and the pharmacokinetics can improve the prescription of simvastatin in combination with other medications and drugs.

Within this thesis the effect of statins on cholesterol metabolism was studied using a computational modelling approach. Specifically, a physiological-based pharmacokinetic model of the statin simvastatin was developed and connected to a model representing hepatic cholesterol synthesis. The model was applied to study the effects of simvastatin on cholesterol biosynthesis and plasma cholesterol levels.

Hypothesis Our main hypothesis was:

The effect of simvastatin on plasma cholesterol levels can be explained by a hepatic model of inhibition of HMG-CoA reductase by active simvastatin metabolites combined with transcriptional/translational regulation of HMG-CoA reductase, HMG-CoA synthase and LDL-receptor mediated cholesterol uptake.

2 Methods

The main methods applied in this thesis were: curation of pharmacokinetic and pharmacodynamics data (sec. 2.1), calculation of pharmacokinetic parameters (sec. 2.2), development of a physiological-based pharmacokinetic/pharmacodynamic model (sec. 2.3), parameter fitting of model parameters (sec. 2.4), uncertainty analysis (sec. 2.5) and sensitivity analysis (sec. 2.6).

2.1 Pharmacokinetics and pharmacodynamics data

2.1.1 Pharmacokinetics database (PK-DB)

PK-DB (https://pk-db.com) is an open database for pharmacokinetics information from clinical studies providing curated information on (i) characteristics of studied patient cohorts and subjects (e.g. age, bodyweight, smoking status); (ii) applied interventions (e.g. dosing, substance, route of application); (iii) measured pharmacokinetic time-courses and (iv) pharmacokinetic parameters (e.g. clearance, half-life, area under the curve) [25].

PK-DB was used as a curation platform and database for the simvastatin and cholesterol pharmacokinetics and -dynamics studies curated in this work. All data presented in this thesis is part of PK-DB.

2.1.2 Curation of data

The data curation process (Fig. 7) consists of multiple steps: literature research for publications of interest, digitization of data and information, upload of data to PK-DB, and checking of data by a second curator.

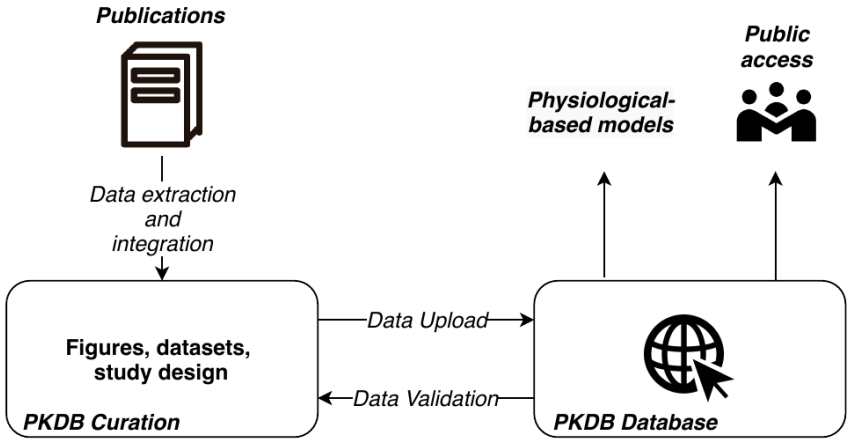


Figure 7: The figure presents the workflow of the curation process for PKDB. Publications are collected and data, such as figures, datasets and information about study design are digitized, stored and uploaded to the PKDB database. This web interface gives instant feedback based on integrated validation rules. The data is free accessible and can be filtered and reviewed or used to create physiological-based models.

The process starts with the research for and the collection of simvastatin and cholesterol publications, based on substance-specific inclusion criteria. The focus for simvastatin were publications reporting the pharmacokinetics of simvastatin and its metabolites after single or multiple dose application. Studies reporting plasma concentration-time courses and pharmacokinetic parameters under control conditions (healthy subjects without the intervention of other drugs) were prioritized.

Inclusion criteria for cholesterol studies were met, if publications reported plasma lipid concentrations (especially LDL-cholesterol) after single or multiple doses of simvastatin. Studies were only included, if it was possible to curate data for baseline and after simvastatin treatment levels of plasma lipids. Therefore either baseline and end concentrations needed to be reported or one of these values with the percentage change. This allowed to calculate the missing corresponding data point. Data for total plasma cholesterol, lipoprotein-cholesterol particles (e.g. VLDL-, IDL-, HDL-cholesterol), triglycerides or cholesterol precursors (e.g. stigmasterol, phytosterol) was curated.

After collection of publications the next step of the curation process is data and information extraction. Concentration-time courses of simvastatin, its metabolites, cholesterol and other lipids were manually digitized (using "PlotDigitizer") and stored with pharmacokinetic parameters in spreadsheets (using "LibreOffice") and standardized JSON-files.

Information about the study design was added to the spreadsheets. This includes data about study subjects and study design. Subject-information includes anthropometrics (e.g. age, height, BMI), medication (e.g. users of oral contraceptives), health status (e.g. any diseases like diabetes etc.) and lifestyle information (e.g. smoking status). Study design includes the administered interventions, characterized by the dose, route (e.g. oral , intravenous), form (e.g. tablet, capsule), the applied substances and the time of administration.

Spreadsheets and JSON data were uploaded to PKDB. During the upload the data is checked against validation rules and sets of constraints, which ensure that all required information is provided in the correct format. Feedback on missing information and curation errors is provided (e.g. missing or wrong units for specific types of measurement). All information is encoded in standardized vocabulary annotated to ontologies. This includes choices for substances, measurement types (e.g. concentration, pharmacokinetic parameters, age or diseases of subjects). After successful upload a second curator checks the data.

2.1.3 Meta-analysis

Pharmacokinetic parameters from multiple studies were analyzed in a meta-analysis. The combination of data from multiple sources allowed to find curation or reporting errors in the data and correct these (e.g. incorrect units). Specifically, the dependency on simvastatin dose was analysed for pharmacokinetics parameters of simvastatin and simvastatin acid. The PK-DB database allowed via simple queries to retrieve all relevant data. Due to standardized encoding of the data and features such as automatic unit conversion the data could be easily combined.

2.2 Pharmacokinetic parameters

Pharmacokinetic parameters were calculated from the simulated concentration-time curves for simvastatin and simvastatin acid using non-compartmental methods. The area under the curve to the end point AUC_{0-end} , the maximal concentration C_{max} and time of maximal concentration t_{max} can directly be calculated from the curve. However the calculation of the area under the curve extrapolated to infinity $AUC_{0-\infty}$, the elimination rate k_{el} , the half-life t_{half} , the volume of distribution V_d , and clearance cl require linear regression of the log-transformed concentration time course.

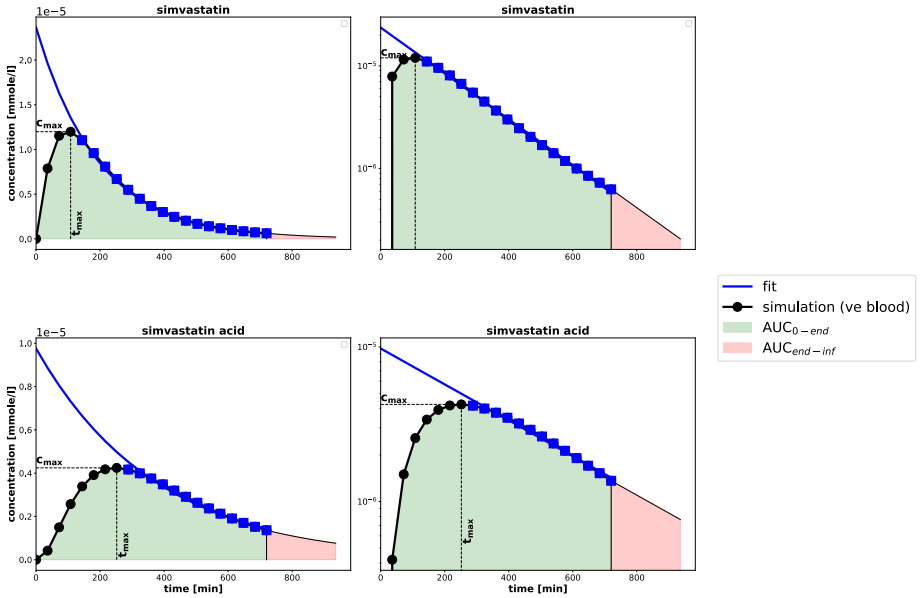


Figure 8: Calculation of pharmacokinetic parameters from concentration time curves. The top panels show simvastatin concentration-time curves, after an application of 40 mg simvastatin orally and the bottom panels the resulting simvastatin acid curves. The right panels are log-transformed. The pharmacokinetic parameters t_{max} and C_{max} are annotated and the area under the curve until the endpoint AUC_{0-end} is shaded in green. The slopes of the log-transformed plots were extrapolated using linear regression to calculate the area under the curve until infinity $AUC_{0-\infty}$.

The area under the curve until the end point AUC_{0-end} and until infinity $AUC_{0-\infty}$ were calculated using the trapezoidal rule. The area-under-the-concentration-curve-area is a measure of the total plasma exposure to the respective substances.

To calculate the parameter k_{el} a linear regression of the log-transformed concentration-time curve after the maximum is performed described by the equation 1. k_{el} is given by the slope and represents the fractional removal rate of the drug from the body. It describes the elimination of the drug and is

independent of the drug dose.

$$log(c(t)) = log(c0) - kel \cdot t \tag{1}$$

The parameter k_{el} can be used to calculate the half-life of the drug t_{half} and the volume of distribution V_d .

The parameter t_{half} describes the removal of a drug by giving the time after which the concentration of the drug reaches half of the maximum concentration C_{max} . It is calculated using equation 2.

$$thalf = \frac{log(2)}{kel} \tag{2}$$

To calculate the volume of distribution equation 3 is applied. The volume of distribution is not a physical volume but a dilution space, describing the volume of the dissolved drug after distribution in the body.

$$vd = \frac{DOSE}{AUC_{inf}} \cdot kel \tag{3}$$

The parameters V_d and k_{el} can be used to calculate the clearance cl with the equation 4. It describes the cleared plasma volume of the drug per time unit.

$$cl = kel \cdot vd \tag{4}$$

Pharmacokinetic parameters were calculated either on curated experimental time courses or on predicted model time courses (e.g. in Fig. 8).

2.3 Pharmacokinetic/pharmacodynamic model (PK/PD)

To develop a pharmacokinetic model of simvastatin and coupled it to a model of cholesterol, an existing framework for physiological-based models on whole-body levels was adapted. This describes the distribution of simvastatin, simvastatin acid and simvastatin metabolites as well as cholesterol on a whole-body scale.

The model allows to simulate the plasma concentrations of simvastatin and simvastatin acid after oral application of simvastatin. Based on the predicted timecourses pharmacokinetic parameters for simvastatin and simvastatin acid were calculated as described in sec. 2.2.

Initial literature research was performed to understand the absorption, distribution, metabolism and excretion of simvastatin and the biological and biochemical pathways of cholesterol biosynthesis and metabolism. The literature research included substance specific biochemical constants, such as Michaelis-Menten constants, inhibitions constants and reference concentration values. Where possible this data was used to parametrize the model. The remaining model parameters were fitted using curated time course data for simvastatin and simvastatin acid (sec. 2.4).

2.3.1 Model representation and simulation

The individual tissue models as well as the whole-body PK/PD-model are ordinary differential equation (ODE) models and can numerically be solved with

ODE solvers. All models were encoded in the Systems Biology Markup Language (SBML) [34, 33], the de facto standard to describe and exchange models in systems biology. All models were completely unit annotated and validated.

The python tool sbmlutils [51] was used to build the model in SBML and the tool sbmlsim [50], which is based on libroadrunner [84] was used as a high-performance simulator to run model simulations.

The model was build using an existing template of a whole-body model which was coupled to tissue-specific models. Within this work tissue models of the liver, intestine and kidney were developed as well as the necessary extensions of the whole-body model to account for simvastatin and cholesterol metabolism. The coupling of tissue models to the whole-body model was performed using the SBML hierarchical model composition [82]. To perform model simulations the model was flattened to create a single SBML model.

2.4 Parameter fitting

Parameter fitting was used to determine parameters for which no experimental data could be found in the literature (sec. 2.3)

The objective of the parameter fitting step was to find values for the parameters so that the resulting time course predictions of simvastatin simvastatin acid, total inhibitors, and active inhibitors are as close as possible to the experimental time courses. The problem can be formulated as an optimization problem which minimizes the residuals between the simulation and data curves. An objective function was defined and minimized using a least square approach using a local optimization strategy [48].

The problem was formulated as a non-linear, bounded-variable least-squares problem. This was solved with SciPy's least_squares method [93]. An objective function was created which follows a L2-norm consisting of the sum of weighted residuals.

The L2-norm can be described with the following equation.

$$cost = 0.5 \cdot \sum (w_k \cdot w_{i,k} \cdot res_{i,k})^2 \tag{5}$$

This formula sums multiple time courses k and time points i. The residual of time point i in time course k is $res_{i,k}$, describing the distance between the data and the model. This distance is weighted with $w_{i,k}$, which weights the data point i in time course k based on the given error of the data point. The time course k is weighted with the factor w_k. In all fittings $w_k = 1.0$ for all k was used weighting all time courses equally.

For each parameter included in the optimization problem constraints as upper and lower bounds were defined. In total 50 sets of initial parameter guesses, sampled from a log-uniform distribution using latin-hypercube sampling within the defined bounds, were used to perform a multi-start least squares fit. The least-square method is a local optimizer using gradient decent. This explores the cost function locally around the initial parameter values.

Fitting of pharmacokinetics model of simvastatin In an initial exploration parameters for the single studies with simvastatin and simvastatin acid timecourses were fitted individually (i.e. for every study a single parameter optimization was formulated). This allowed to test if the individual studies can

be described with the developed model. The set of parameter fits resulted in a parameter range for each fitting parameter in which it describes the model the best. These parameter bounds were subsequently used to constrain the parameter ranges for the fit of all studies. The resulting optimal parameters were used in the reference model.

The fitted parameters for the simvastatin model included distribution parameters for the different substances, absorption parameters for simvastatin and maximal velocities for the Michael-Menten kinetics for the transport and biochemical reactions in the intestine and liver.

2.5 Uncertainty analysis

An uncertainty analysis was performed for most model simulations to quantify the uncertainty depending on model parameters of predicted time courses and calculated pharmacokinetic parameters.

Model simulations were performed varying each parameter individually by $\pm$ 50% resulting in a set of time course predictions. From the set of simulations the standard deviation (SD) and the maximum and minimum values for each time point were calculated and displayed in the plots as shaded areas. This allowed to evaluate the variability in model predictions when changing all model parameters systematically. Furthermore robustness of the model around the reference parameter set could be tested.

Pharmacokinetic parameters were calculated (sec. 2.2) for the complete set of time course predictions and standard deviations (SD) were calculated.

Parameters for physical constants (such as molecular weights), conversion factors, and dosing were excluded from the variation for the uncertainty analysis.

2.6 Sensitivity analysis

The effect of model parameters on model behaviour was analysed using sensitivity analysis. Simulation experiments were set up and throughout model parameters were varied and the resulting changes in model outputs quantified.

For the sensitivity analysis of pharmacokinetic parameters, individual model parameters p_i were changed by 10% from their reference value ($p_{i,0} \longrightarrow p_{i,\Delta}$). The sensitivity was then calculated by normalizing the change in the pharmacokinetic parameter from baseline with the parameter change. The sensitivity $S(q_k, p_i)$ of the pharmacokinetic parameter q_k with respect to model parameter p_i is calculated with equation 6.

$$S(q_k, p_i) = \frac{q_k(p_{i,0}) - q_k(p_{i,\Delta})}{p_{i,0} - p_{i,\Delta}} \tag{6}$$

Parameters for physical constants (such as molecular weights), conversion factors, and dosing were excluded from the variation for the sensitivity analysis.

Pharmacokinetic parameters for simvastatin, simvastatin acid and simvastatin metabolites were calculated and the effect on the varied model parameters evaluated based on outputs of the sensitivity-timecourses.

Analogically to the sensitivity analysis for pharmacokinetic parameters, model readouts of the cholesterol model were analysed. Here, no pharmacokinetic parameters were calculated, but readouts such as concentrations for LDL-cholesterol or protein levels were analysed. Due to the time dependence of those readouts

the mean concentration of one single day was calculated. The sensitivity represents the change in such readouts compared to simulations without varied parameters.

3 Results

Within this work a pharmacokinetic/pharmacodynamic (PK/PD) model of simvastatin including the effects on HMG-CoA reductase activity and plasma cholesterol levels was developed. The model was calibrated and evaluated using data curated from the literature (sec. 3.1). Within this section the model for simvastatin pharmacokinetics and cholesterol pharmacodynamics are presented, including details about model parametrization (sec. 3.3). The simvastatin model was validated by comparing model predictions to timecourse and pharmacokinetics data of simvastatin and its metabolites (sec. 3.4). The coupled simvastatin and cholesterol model was applied to study the effects of simvastatin on HMG-CoA reductase activity and plasma LDL-cholesterol levels (sec. 3.5).

3.1 Simvastatin and cholesterol data

3.1.1 Data curation

Within this work a large database of time courses and pharmacokinetics data of simvastatin as well as pharmacodynamics data in simvastatin therapy (total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides) was established. Importantly, multiple studies reporting effects of simvastatin therapy on plasma LDL-cholesterol under varying dose and duration were part of the curation effort.

Overall data from more than 40 studies was curated with an overview of the studies given in Tab. 1. All data is freely available via the pharmacokinetics database PK-DB [25]. The overview of the curated simvastatin pharmacokinetics parameters are given in Tab. 2 and Tab. 3. The overview of time courses of simvastatin and its metabolites is provided in Tab. 4. The overview of time courses reporting changes in cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides is provided in Tab. 5. To our knowledge this is the largest freely available data collection related to simvastatin pharmacokinetics.

The curated database was used in developing and evaluating the PK/PD model: (i) time courses of plasma simvastatin, simvastatin acid, total inhibitors and active inhibitors after single dose application of simvastatin were used for simvastatin model parametrization (sec. 3.3.1) and after multiple dose application to validate model predictions (sec. 3.4.1); (ii) pharmacokinetics parameters of simvastatin and multiple dose administration timecourses were used for model validation (sec. 3.4.2); and (iii) pharmacodynamic data (changes in LDL-cholesterol due to simvastatin therapy) were used for evaluating predicted effects on plasma LDL-cholesterol levels (sec. 3.5.2 and sec. 3.5.4).

Table 1: Overview of curated studies. For each study the table shows which information was reported. Either, information was reported (✓), partially reported (⊘) or not reported at all (whitespace).

PKDB	name	subjects	groups	sex	age	weight	height	bmi	ethnicity	healthy	medication	smoking	fast	alcohol	dose	route	form	method
PKDB00345	Backman2000	10	1	✓	⊘	⊘				✓	✓	✓	✓		⊘	⊘	⊘	✓
PKDB00361	Bergman2004	12	1	⊘						⊘	⊘							✓
PKDB00351	Cheng1992	34	4	⊘						⊘	⊘							✓
PKDB00367	Cheng1994	4	1							✓			✓		✓	✓	✓	✓
PKDB00243	Chung2006	19	6	⊘	⊘	⊘		⊘	⊘	⊘					✓	✓	✓	
PKDB00352	Gehin2015	22	1	✓	✓			✓	✓	✓	✓	✓	✓		✓	✓	✓	✓
PKDB00356	Harvey2018	57	1	⊘	⊘	⊘	⊘	⊘	⊘	⊘					✓	✓	✓	✓
PKDB00355	Hsyu2001	31	2	✓	✓	✓			✓	✓	✓	✓						✓
PKDB00364	Jacobson2004	30	2	⊘						⊘	⊘				⊘	⊘	⊘	✓
PKDB00366	Jiang2017	26	5						⊘	⊘	⊘		⊘	⊘	✓	✓	✓	✓
PKDB00296	Jones1998a	518	13	⊘	⊘					⊘	⊘							
PKDB00350	Kantola1998	12	1	✓	⊘	⊘				✓	✓	✓			✓	✓	✓	✓
PKDB00353	Kim2019	19	1	⊘	⊘	⊘	⊘	⊘		⊘	⊘		⊘		✓	✓	✓	✓
12236852	Kosoglou2002	82	8	⊘	⊘	⊘				⊘	⊘		⊘					
PKDB00365	Kyrklund2000	10	1	✓						✓	✓	✓	✓		✓	✓	✓	✓
PKDB00342	Lilja1998	10	1	⊘						⊘	⊘	⊘			✓	✓	✓	✓
PKDB00354	Lilja2000	10	1	⊘						⊘	⊘	⊘			✓	✓	✓	
PKDB00344	Lilja2004	10	1	⊘						⊘	⊘	⊘	⊘		✓	✓	✓	✓
PKDB00346	Lohitnavy2004	18	1	⊘	⊘			⊘	⊘	⊘	⊘	⊘	⊘		✓	✓	✓	✓
PKDB00373	Loria1993	5	1	⊘	⊘	⊘	⊘			⊘	⊘		⊘		✓	✓	✓	
PKDB00360	Marino2000	14	1	⊘	⊘	⊘	⊘		⊘	⊘	⊘		⊘		✓	✓	✓	✓
PKDB00362	Mousa2000	10	1	✓	✓	✓				✓	✓	✓	✓		⊘	⊘	⊘	✓
PKDB00349	Najib2003	24	1	⊘	⊘	⊘	⊘			⊘	⊘		⊘		✓	✓	✓	✓
PKDB00372	Neuvonen1998	20	1	✓	✓	✓				✓	✓	✓			✓	✓	✓	⊘
PKDB00375	Ntanios1999	18	5	⊘	⊘	⊘	⊘	⊘		⊘								
PKDB00369	OBrien2003	20	1	✓	✓					✓	✓				✓	✓	✓	✓
PKDB00368	Pasanen2006	32	3	✓	✓	✓	✓		✓	✓	✓	✓	✓		✓	✓	✓	⊘
PKDB00371	Pentikainen1992	12	1	⊘	⊘	⊘				⊘	⊘	⊘	⊘		✓	✓	✓	
PKDB00370	Prueksaritanont2001	24	3	✓						✓	✓	✓						✓
PKDB00358	Simard2001	11	1	✓						✓	✓	✓	✓					✓
PKDB00359	Sugimoto2001	16	2	✓	✓	✓			✓	✓	✓	✓	✓		✓	✓	✓	✓
PKDB00343	TubicGrozdanis2008	7	1	⊘	⊘	⊘				⊘	⊘	⊘			✓	✓	✓	✓
PKDB00374	Tuomilehto1995	166	6	⊘	⊘				⊘	⊘								
PKDB00347	Ucar2004	12	1	⊘					⊘	⊘	⊘				✓	✓	✓	✓
Walker1990	Walker1990	160	5	⊘	⊘					⊘								
PKDB00357	Zhi2003	29	1	⊘	⊘	⊘	⊘	⊘	⊘	⊘		⊘			✓	✓	✓	✓
PKDB00363	Zhou2013	17	25	⊘					⊘	⊘	⊘	⊘	⊘		✓	✓	✓	✓
PKDB00348	Ziviani2001	18	1	⊘	⊘	⊘			⊘	⊘	⊘	⊘						✓

Table 2: Overview of curated pharmacokinetics parameters. For each study the table shows which pharmacokinetics parameters of simvastatin (S), simvastatin acid (SA), simvastatin active inhibitors (AI), simvastatin total inhibitors (TI) and simvastatin + simvastatin acid (S + SA) were reported. Whether a parameter was reported for individuals, groups and whether an error (SD or SE) was reported is shown as a triplet (in this order). Either, a parameter was reported for all individuals/groups (✓), only for some individuals/groups (⊘) or not reported (empty).

		auc					clearance					cmax					kel				
PKDB	name	S	SA	AI	TI	S+SA	S	SA	AI	TI	S+SA	S	SA	AI	TI	S+SA	S	SA	AI	TI	S+SA
PKDB00345	Backman2000	✓✓	✓✓				✓					✓✓	✓✓				✓	✓			
PKDB00361	Bergman2004	✓✓	✓✓	✓✓	✓✓							✓✓	✓✓	✓✓	✓✓						
PKDB00351	Cheng1992			✓✓	✓✓									✓✓	✓✓						
PKDB00367	Cheng1994				✓										✓					✓	
PKDB00243	Chung2006	⊘					✓					⊘					⊘				
PKDB00352	Gehin2015	✓✓	⊘✓									⊘✓	✓✓				✓	✓			
PKDB00356	Harvey2018	✓	✓				✓✓					✓	✓				✓	✓			
PKDB00355	Hsyu2001			✓										✓							
PKDB00364	Jacobson2004	✓	✓				⊘					✓	✓				⊘	⊘			
PKDB00366	Jiang2017	✓⊘	✓⊘				⊘					✓⊘	✓⊘				⊘	⊘			
PKDB00350	Kantola1998	✓✓	✓✓									✓✓	✓✓					✓			
PKDB00353	Kim2019	✓✓	✓✓				✓					✓✓	✓✓				✓	✓			
12236852	Kosoglou2002	✓✓	✓✓				✓✓					✓✓	✓⊘								
PKDB00365	Kyrklund2000	✓✓✓	✓✓✓				✓					✓✓	✓✓				✓	✓			
PKDB00342	Lilja1998	✓✓	✓✓	✓✓	✓✓		✓					✓✓	✓✓	✓✓	✓✓		✓	✓	✓	✓	
PKDB00354	Lilja2000	✓	✓				✓					✓	✓				✓	✓			
PKDB00344	Lilja2004	✓✓	✓✓				✓					✓✓	✓✓				✓	✓			
PKDB00346	Lohitnavy2004	✓✓		✓✓			✓					✓✓		✓✓			✓		✓		
PKDB00360	Marino2000					✓✓										✓✓					✓
PKDB00362	Mousa2000	✓✓✓	⊘				✓					✓✓✓	✓✓✓				✓				
PKDB00349	Najib2003	✓✓	✓✓				✓					✓✓	✓✓				✓✓	✓✓			
PKDB00372	Neuvonen1998	✓			✓✓	✓✓✓						✓			✓✓	✓✓✓				✓	✓
PKDB00369	OBrien2003	✓✓✓	✓✓				✓✓					✓✓	✓✓				✓	✓			
PKDB00368	Pasanen2006	⊘✓✓	⊘✓✓				✓					✓✓✓	✓✓✓				✓	✓			
PKDB00371	Pentikainen1992			✓✓	✓✓									✓✓	✓✓				✓	✓	
PKDB00370	Prueksaritanont2001			✓⊘⊘	✓⊘⊘									⊘⊘	⊘⊘						
PKDB00358	Simard2001	✓✓✓	✓✓✓									✓✓	✓✓								
PKDB00359	Sugimoto2001	✓✓✓	✓✓✓									✓✓✓	✓✓✓					✓			
PKDB00343	TubicGrozdanis2008	✓✓	✓✓				✓✓	✓✓				✓✓	✓✓				✓✓	✓			
PKDB00347	Ucar2004	✓✓	✓✓				✓					✓✓	✓✓				✓	✓			
PKDB00357	Zhi2003	✓	✓				✓					✓	✓				✓	✓			
PKDB00363	Zhou2013	✓✓					⊘					✓✓					⊘				
PKDB00348	Ziviani2001					✓✓										✓✓					

Table 3: Overview of curated pharmacokinetics parameters (continued).

		thalf					tmax					vd				
PKDB	name	S	SA	AI	TI	S+SA	S	SA	AI	TI	S+SA	S	SA	AI	TI	S+SA
PKDB00345	Backman2000	✓✓	✓✓				✓	✓				✓				
PKDB00361	Bergman2004						✓	✓	✓	✓						
PKDB00351	Cheng1992								✓✓	✓✓						
PKDB00367	Cheng1994				✓					✓						
PKDB00243	Chung2006	⊘					⊘					⊘				
PKDB00352	Gehin2015	✓	✓				✓	✓								
PKDB00356	Harvey2018	✓✓	✓✓				✓	✓				✓✓				
PKDB00355	Hsyu2001								✓							
PKDB00364	Jacobson2004	⊘	⊘				⊘	⊘				⊘				
PKDB00366	Jiang2017	✓⊘	✓⊘				⊘	⊘				⊘				
PKDB00350	Kantola1998	✓✓	✓✓				✓	✓								
PKDB00353	Kim2019	✓✓	✓✓				✓	✓				✓				
12236852	Kosoglou2002	✓✓	✓✓				✓	✓				✓✓				
PKDB00365	Kyrklund2000	✓✓	✓✓				✓	✓				✓				
PKDB00342	Lilja1998	✓✓	✓✓	✓✓	✓✓		✓	✓	✓	✓		✓				
PKDB00354	Lilja2000	✓	✓				✓	✓				✓				
PKDB00344	Lilja2004	✓✓	✓✓				✓	✓				✓				
PKDB00346	Lohitnavy2004	✓✓		✓✓			✓✓		✓✓			✓				
PKDB00360	Marino2000					✓✓					✓✓					
PKDB00362	Mousa2000	✓✓✓					✓✓✓	✓✓✓				✓				
PKDB00349	Najib2003	✓✓	✓✓				✓✓	✓✓				✓				
PKDB00372	Neuvonen1998				✓✓	✓✓✓	✓			✓✓	✓✓✓					
PKDB00369	OBrien2003	✓✓	✓✓				✓	✓				✓✓				
PKDB00368	Pasanen2006	⊘✓✓	⊘✓✓				✓	✓				✓				
PKDB00371	Pentikainen1992			✓	✓				✓✓	✓✓						
PKDB00370	Prueksaritanont2001								⊘⊘	⊘⊘						
PKDB00358	Simard2001						✓✓	✓✓								
PKDB00359	Sugimoto2001		✓				✓✓✓	✓✓✓								
PKDB00343	TubicGrozdanis2008	✓✓	✓✓				✓✓	✓✓				✓✓	✓✓			
PKDB00347	Ucar2004	✓✓	✓✓				✓	✓				✓				
PKDB00357	Zhi2003	✓	✓				✓	✓				✓				
PKDB00363	Zhou2013	✓✓					⊘⊘					⊘				
PKDB00348	Ziviani2001					✓✓					✓✓					

Table 4: Overview of all curated simvastatin time courses. The table shows for each study whether time course data for simvastatin (S), simvastatin acid (SA), simvastatin active inhibitors (AI), simvastatin total inhibitors (TI) and simvastatin + simvastatin acid (S + SA) was reported. The table further shows whether the time course data was reported for single individuals, groups, whether an error (SD or SE) was reported and the site of measurement (plasma/blood/serum, urine and saliva). Either, time course data (or errors) was reported for all individuals/groups (✓), only for some individuals/groups (⊘) or not reported (empty).

		simvastatin (S)					simvastatin acid (SA)					active inhibitors (AI)					total inhibitors (TI)					S+SA				
PKDB	name	individual	group	error	plasma	urine	individual	group	error	plasma	urine	individual	group	error	plasma	urine	individual	group	error	plasma	urine	individual	group	error	plasma	urine
PKDB00345	Backman2000		✓	✓	✓			✓	✓	✓																
PKDB00361	Bergman2004		✓		✓			✓		✓			✓		✓			✓		✓						
PKDB00367	Cheng1994																✓	✓	✓	✓	✓					
PKDB00243	Chung2006		✓	✓	✓																					
PKDB00352	Gehin2015		✓	✓	✓			✓	✓	✓																
PKDB00356	Harvey2018		✓	✓	✓			✓	✓	✓																
PKDB00355	Hsyu2001												✓	✓	✓											
PKDB00364	Jacobson2004		✓		✓			✓		✓																
PKDB00366	Jiang2017		✓		✓			✓		✓																
PKDB00350	Kantola1998		✓	✓	✓			✓	✓	✓																
PKDB00353	Kim2019		✓	✓	✓			✓	✓	✓																
PKDB00365	Kyrklund2000		✓	✓	✓			✓	✓	✓																
PKDB00342	Lilja1998		✓	✓	✓			✓	✓	✓			✓	✓	✓			✓	✓	✓						
PKDB00354	Lilja2000		✓		✓			✓		✓																
PKDB00344	Lilja2004		✓	✓	✓			✓	✓	✓																
PKDB00346	Lohitnavy2004		✓		✓								✓		✓											
PKDB00360	Marino2000																						✓		✓	
PKDB00362	Mousa2000		✓	✓	✓																					
PKDB00349	Najib2003		✓	✓	✓			✓	✓	✓																
PKDB00372	Neuvonen1998		✓	✓	✓													✓	✓	✓			✓	✓	✓	
PKDB00369	OBrien2003		✓	✓	✓			✓	✓	✓																
PKDB00368	Pasanen2006		✓	✓	✓			✓	✓	✓																
PKDB00371	Pentikainen1992												✓		✓			✓		✓						
PKDB00370	Prueksaritanont2001												✓	✓	✓			✓	✓	✓						
PKDB00358	Simard2001		✓	✓	✓			✓	✓	✓																
PKDB00359	Sugimoto2001		✓	✓	✓			✓	✓	✓																
PKDB00343	TubicGrozdanis2008		✓	✓	✓			✓	✓	✓																
PKDB00347	Ucar2004		✓	✓	✓			✓	✓	✓																
PKDB00357	Zhi2003		✓		✓			✓		✓																
PKDB00363	Zhou2013		✓	✓	✓																					
PKDB00348	Ziviani2001																						✓	✓	✓	

Table 5: Overview of all curated cholesterol time courses. The table shows for each study whether time course data for cholesterol, LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), and triglycerides was reported. The table further shows whether the time course data was reported for single individuals, groups, whether an error (SD or SE) was reported and the site of measurement (plasma/blood/serum). Either, time course data (or errors) was reported for all individuals/groups (✓), only for some individuals/groups (⊘) or not reported (empty).

		cholesterol				LDL-C				HDL-C				triglyceride			
PKDB	name	individual	group	error	plasma	individual	group	error	plasma	individual	group	error	plasma	individual	group	error	plasma
PKDB00296	Jones1998a		✓	✓	✓		✓	✓	✓		✓	✓	✓		✓	✓	✓
12236852	Kosoglou2002		✓	✓	✓		✓	✓	✓		✓	✓	✓		✓	✓	✓
PKDB00373	Loria1993		✓	✓	✓		✓	✓	✓		✓	✓	✓				
PKDB00375	Ntanios1999		✓	✓	✓		✓	✓	✓		✓	✓	✓		✓	✓	✓
PKDB00374	Tuomilehto1995		✓		✓		✓		✓		✓		✓		✓		✓
Walker1990	Walker1990		✓		✓						✓		✓				

3.1.2 Meta analysis

As part of the work pharmacokinetics data was combined in the form of a meta-analysis. This allows to detect and correct outliers in the data (due to curation errors or reporting problems) and to examine the dose-dependency of simvastatin and simvastatin acid pharmacokinetics.

The data for the meta-analysis was filtered to only include data after single oral application of simvastatin in healthy subjects. Data sets studying drug-drug interactions by applying additional drugs or subjects with co-medication as well as data in subjects with reported diseases were excluded.

A representative example of the resulting meta-analysis is shown in Fig. 9.

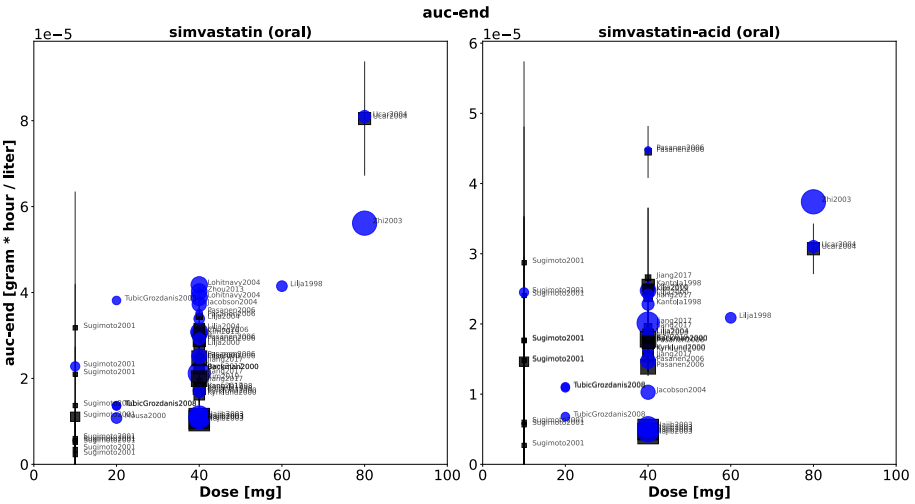


Figure 9: Meta-analysis of the dose-dependency of AUC_{0-end} of simvastatin (left) and simvastatin acid (right). Data points are either directly from curated publications (blue) or calculated from digitized time-course data (black). Data is mean ± SE where errors are provided.

The meta-analysis provided information about the dose-dependency of simvastatin and simvastatin. It can be seen that the AUC_{0-end} of simvastatin and simvastatin acid increases linearly with the simvastatin dose.

Information from the pharmacokinetics meta-analysis was used to validate model predictions of the simvastatin pharmacokinetics model. Corresponding data for other pharmacokinetics parameters is presented in the respective section in the supplement (sec. 3.4.2).

3.2 Computational model

Within this section the developed pharmacokinetic model of simvastatin (sec. 3.2.1) and pharmacodynamic model of the effects of simvastatin on HMG-CoA reductase activity and plasma cholesterol (sec. 3.2.2) are presented.

Kinetics Biochemical reactions and transport reactions were modelled using either reversible or irreversible Michaelis-Menten kinetics or reaction of zero or first order. If not stated otherwise reactions and rates follow one of these four types.

Irreversible Michaelis-Menten kinetics were described via

$$v = \frac{V_{max} \cdot V \cdot S}{K_m}$$

and reversible via

$$v = \frac{\frac{V_{max}}{K_m} \cdot V \cdot (S - P)}{1 + \frac{P}{K_m} + \frac{S}{K_m}}$$

Each of the two Michaelis-Menten kinetics depend on the maximal velocity V_{max} and the Michaelis-Menten constant K_m. Reversible Michaelis-Menten Kinetics were mainly used for transporters with K_m for substrate and product assumed identical. S and P are the concentrations of the substrate and product, respectively.

Reactions of zero order (constant rate) were described via

$$v = k \cdot V$$

and reactions of first order were described via

$$v = k \cdot V \cdot S$$

The parameter k is the respective rate constant and S is the concentration of the substrate. All reactions were scaled with a volume factor V to allow scaling with changing organ volumes. Depending on the tissue and type of reaction the rates were scaled either on a volume factor (e.g. liver volume) or total body mass (e.g. body weight).

3.2.1 Pharmacokinetic model of simvastatin

Whole-body model The basis of the physiological-based model of simvastatin pharmacokinetics was an existing template of a whole-body model (unpublished work). The template-model allows to describe the absorption, distribution, excretion and systemic circulation of substances in the body. The various tissues are modelled as compartments which are connected via the blood flow. Physiological parameters of the model are defined as part of the whole-body model including volumes of the organs, blood flows describing the perfusion of organs, body weight, or cardiac output. The whole-body template was adapted for simvastatin (Fig. 10).

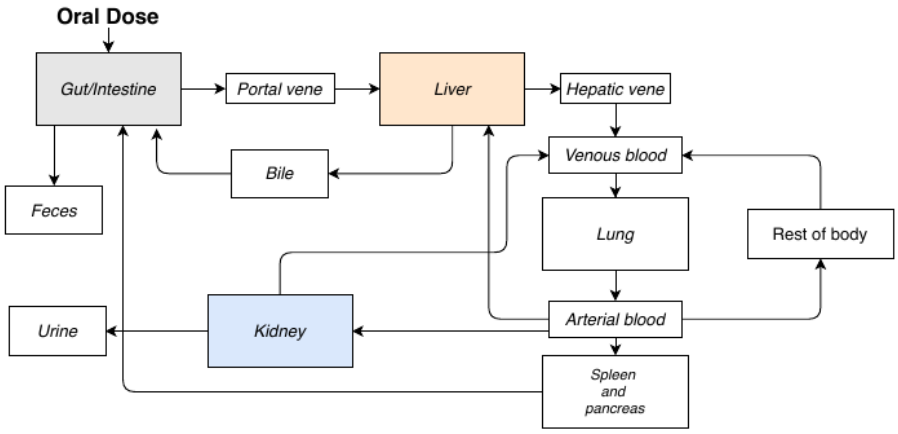


Figure 10: Overview of organs and systemic circulation in the whole-body model after oral application of simvastatin. The drug is absorbed by the gut/intestine reaching the liver via the portal vein. A fraction of the dose is excreted into the feces. After metabolization in the liver simvastatin or its metabolites can either be transported to the hepatic vein or undergo enterohepatic circulation, i.e., being exported from the liver through the bile back to the gut. Venous blood flows through the lungs, is oxygenated and becomes arterial blood. From the arterial blood the substances can reach the various organs such as the liver, spleen, pancreas, gut, kidney or rest of the body (remaining organs). After passing the organs the blood becomes venous blood. The kidney can excrete simvastatin metabolites into the urine.

Simvastatin is absorbed by the gut/intestine reaching the liver via the portal vein. The unabsorbed fraction of the simvastatin dose is excreted into the feces. After metabolization in the liver, simvastatin and its metabolites can either be transported to the hepatic vein or undergo enterohepatic circulation, i.e., being exported from the liver through the bile back to the gut. Venous blood flows through the lungs, is oxygenated and becomes arterial blood. From the arterial blood the substances can reach the various organs such as the liver, spleen, pancreas, gut, kidney or rest of the body (remaining organs). The kidney can excrete simvastatin metabolites into the urine.

The pharmacokinetics model of simvastatin includes three main metabolites: (i) simvastatin, (ii) the main active metabolite simvastatin acid, and (iii) so-called simvastatin metabolites. As discussed in the introduction (sec. 1.2.1) simvastatin and simvastatin acid undergo conversion by CYP3A4 to various secondary metabolites. Within the model the simvastatin metabolites account for all of these secondary metabolites.

Tissue models of the organs relevant for simvastatin pharmacokinetics were created and connected to the whole body template, i.e. for the liver, gut/intestine and kidney.

The absorption kinetics of simvastatin play an important role for the overall pharmacokinetics of simvastatin after oral dosing. Dissolution of simvastatin and transport through the stomach introduce a time delay with which orally ap-

plied drugs appear in the intestine before they can be absorbed. These processes were lumped in a single reaction of first order (equation 7).

$$v_{dissolution} = ka_{dis} \cdot \frac{PODOSE}{M_R} \tag{7}$$

The velocity of the dissolution depends on the oral dose ($PODOSE$) and the parameter ka_{dis}, describing the rate of dissolution/ stomach passage. The molecular weight of simvastatin M_R is used to convert between applied doses in mg and substance units used in the model in mmole. The amount of dissolved dose is tracked via the state variable $GUTDOSE$, describing the amount of dose already dissolved in the intestine, but not yet absorbed.

Subsequent absorption of simvastatin by the gut was modelled as first order reaction (equation 8).

$$v_{absorption} = ka_{abs} \cdot \frac{GUTDOSE}{M_R} \tag{8}$$

The absorption in the gut/intestine depends on the absorption rate ka_{abs} with which the $GUTDOSE$ is absorbed.

The resulting differential equations for the dissolution and absorption are

$$\frac{dPODOSE}{dt} = -v_{dissolution} \cdot M_R \tag{9}$$

$$\frac{dGUTDOSE}{dt} = F_{simvastatin} \cdot v_{dissolution} \cdot M_R - v_{absorption} \cdot M_R \tag{10}$$

The fraction $F_{simvastatin}$ of the dissolved dose is absorbed by the gut, the remaining fraction is excreted as unabsorbed simvastatin into the feces via equation 11.

$$v_{feces_{simvastatin}} = (1 - F_{simvastatin}) \cdot v_{dissolution} \tag{11}$$

The rate depends on the velocity of the dissolution reaction (equation 7) and fraction absorbed $F_{simvastatin}$. One study reported this fraction as approximately 60% [8], while another study reported 61-85% [90]. Based on these results and very low reported simvastatin in the feces (based on radioactive tracers) a fraction absorbed of 0.85 was used in the model (85% of a dose are absorbed and 15% are directly excreted into the feces).

After absorption by the gut, simvastatin can either be metabolized there or transported via the portal vein to the liver where further metabolization can take place. From the liver simvastatin and its metabolites can reach the systemic circulation. The kidney can remove substances from the blood. The corresponding tissue models of the intestine, liver and kidney are described below.

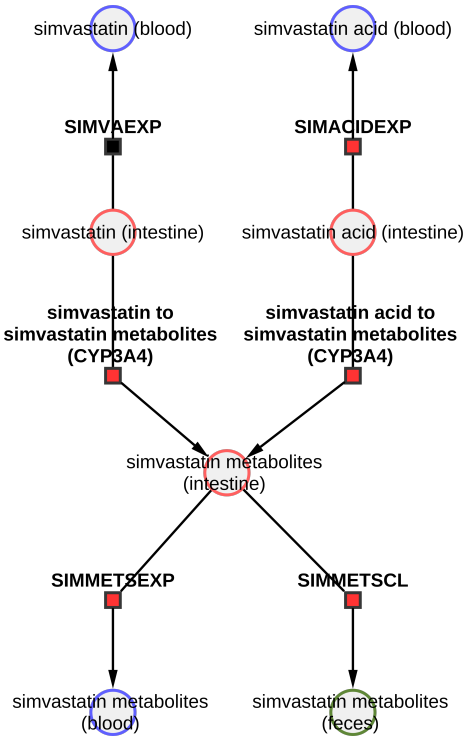


Figure 11: Overview of the intestinal tissue model. Metabolites are depicted as circles and reactions as squares (red irreversible, black reversible). Simvastatin, simvastatin acid and simvastatin metabolites can be exchanged between the intestine and the blood. Simvastatin and simvastatin acid can be metabolized via CYP3A4 in the intestine to simvastatin metabolites. The simvastatin metabolites can be excreted in feces by the intestine. The plot was created using CySBML [46].

Intestine submodel Substances taken up orally are reaching first the stomach and subsequently the intestine. From the intestinal content the substances can be absorbed by the intestine and are transported in the portal vein.

The exchange of simvastatin between blood and gut is modelled via a reversible Michaelis-Menten kinetic. Because of the reversibility, simvastatin can be taken up by the intestine, but is also able to be excreted into the blood.

Furthermore the intestinal submodel includes two biochemical reactions corresponding to the first-pass metabolism through CYP3A4-enzymes in the intestinal wall. These reactions are the conversion from simvastatin and simvastatin acid to simvastatin metabolites, modelled with irreversible Michaelis-Menten kinetics. Simvastatin acid and simvastatin metabolites can only be exported from the intestine into the plasma blood (irreversible Michaelis-menten kinetics).

The fecal excretion was modelled with a kinetic of first order. Only simvastatin metabolites can be excreted based on the fact that little or no simvastatin and simvastatin acid have been reported in the feces after simvastatin administration (see sec. 1.2.2). Once excreted, substances can not reappear in the

systemic circulation, but are removed from the body.

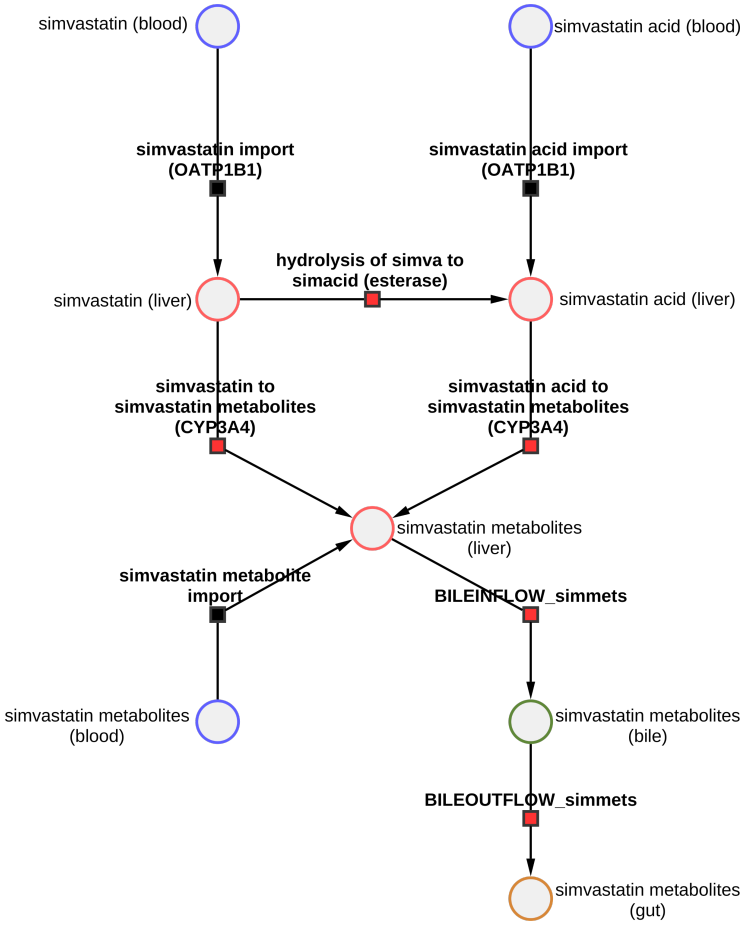


Figure 12: Overview of the liver tissue model. Metabolites are depicted as circles and reactions as squares (red irreversible, black reversible). Simvastatin, simvastatin acid and simvastatin metabolites can be exchanged between the liver and the blood. Simvastatin and simvastatin acid can be metabolized via CYP3A4 in the liver to simvastatin metabolites. Simvastatin can be converted by esterases to simvastatin metabolites in the liver. The simvastatin metabolites can be transported via the bile to the gut/intestine via enterohepatic circulation. The plot was created using CySBML [46].

Liver model Blood from the intestine reaches the liver via the portal vein. Simvastatin, simvastatin acid and simvastatin metabolites can be reversibly exchanged between the blood and the liver. Reversible Michaelis-Menten kinetics were used to describe the OATP1B1 mediated import.

The liver is besides the intestine the major site of simvastatin metabolism (Fig. 12). To model the hepatic metabolism, three biochemical reactions were implemented. The first reaction is the activation of simvastatin to simvastatin

acid mediated by esterases. The remaining two reactions are similar to the biochemical reactions in the intestinal submodel. These are the conversion of simvastatin and simvastatin acid to simvastatin metabolites catalyzed by CYP3A4. All three reactions are modelled with irreversible Michaelis-Menten kinetics.

As mentioned in the introduction simvastatin and its metabolites are subjects to enterohepatic circulation via the bile to the gut. The model assumption was that only simvastatin metabolites undergoes enterohepatic circulation (based on the fact that only very low amounts of simvastatin and simvastatin acid are found in the bile). The transport into the bile and from the bile into the gut were modelled with first-order kinetics.

Kidney model The kidney plays a role in the excretion of simvastatin derived metabolites. Excretion in the urine was modelled via a first order kinetics that transports simvastatin metabolites from the arterial blood irreversibly to the urine.

$$v_{SIMMETSCL} = k \cdot f \cdot V_{ki} \cdot simmets_{ext} \tag{12}$$

The velocity of the urinary clearance depends on the parameter for simvastatin metabolite clearance k and f, which correspond to the model parameters $SIMMETSCL\_k$ and $SIMMETSCL\_f$. The parameter $SIMMETSCL\_f$ determines the fraction of the urinary clearance in comparison to the fecal clearance. Introducing a global clearance parameter $SIMMETSCL\_k$ in combination with a fractional clearance parameter allowed to restrict the contribution of urinary excretion within the parameter fitting. Findings that the fecal and urinary clearance account for approximately 60% and 10%, respectively, could so be used as an additional restriction in parameter fitting.

As a model simplification the transport reactions from the plasma to the kidneys and from the kidneys to the urine were lumped as a single direct transport reaction from the plasma into the urine with renal excretion depending on the plasma concentration $simmets_{ext}$ of simvastatin metabolites. As for all tissue reactions scaling with the respective organ volume was performed, here with the kidney volume V_{ki}.

Simvastatin and simvastatin acid are not excreted in the urine, based on the findings that little or no unchanged simvastatin or simvastatin acid can be found in the urine (sec. 1.2.2).

3.2.2 Pharmacodynamic model of cholesterol

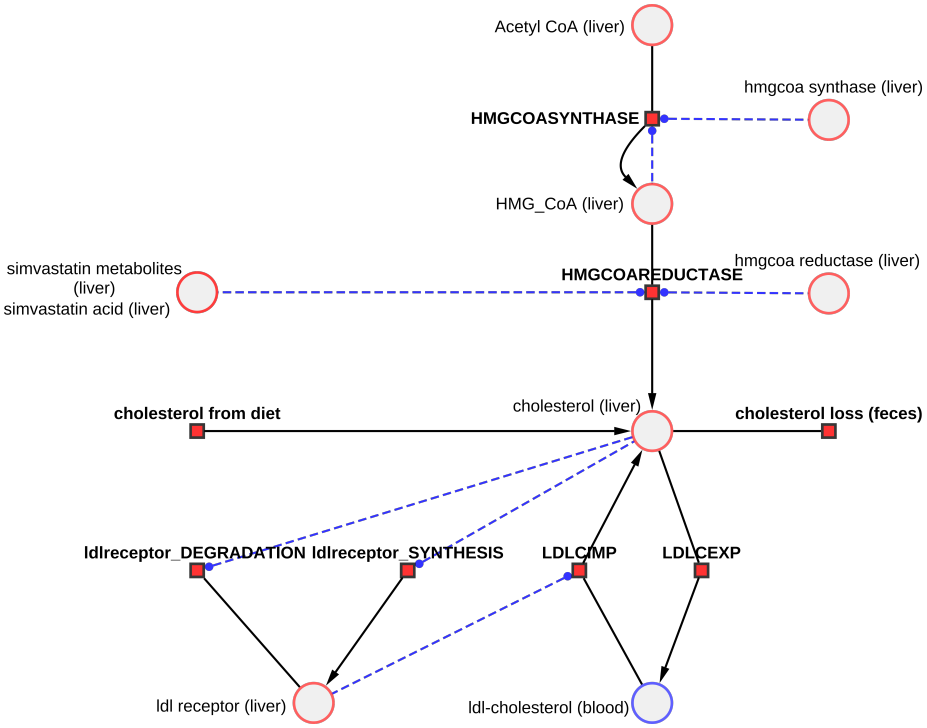


Figure 13: Overview of cholesterol homeostasis and biosynthesis in the liver as modelled. Metabolites are depicted as circles and reactions as squares (red irreversible, black reversible). A simplified model of cholesterol biosynthesis from acetyl-CoA via the two main reactions HMG-CoA synthase and HMG-CoA reductase were implemented. Blue dotted lines represent modulation such as inhibition or induction of a reaction by a substance. As an example the hmgcoa synthase (liver) inhibits the HMGCOASYNTHASE kinetic via product inhibition. The regulation of ldlreceptor_DEGRADATION and -SYNTHESIS kinetics via hepatic cholesterol are important for ldlreceptor (liver) levels. Additionally the same kinetics of modulation mediated by cholesterol (liver) are implemented for the HMG-CoA reductase (liver) and HMG-CoA synthase (liver), but not shown. The plot was created using CySBML [46].

To examine the pharmacodynamics of simvastatin a simplified model of cholesterol homeostasis was developed (Fig. 13). This model is mainly located in the liver submodel and focuses on processes that are relevant for maintain hepatic cholesterol homeostasis. The *de novo* synthesis, the uptake and export of plasma LDL-cholesterol, the uptake from dietary sources and the excretion in the feces as bile acids or unchanged cholesterol.

Regulation of protein amounts Cholesterol metabolism in the liver is highly regulated by feedback mechanisms via cholesterol. Hepatic cholesterol

levels regulate the expression and degradation of the proteins HMG-CoA reductase, HMG-CoA synthase and the LDL-receptor. These proteins were implemented as hmgcoareductase, hmgcoasynthase and ldlreceptor variables in the liver model. Two reactions affect these protein amounts, one describing the expression/translation, the other describing the degradation. Both reactions were modeled as zero order kinetics with an additional regulatory term depending on hepatic cholesterol (equation 13 and 14).

$$v_{synthesis} = k_{synthesis} \cdot Vli \frac{1}{1 + \frac{cho}{kr}} \tag{13}$$

$$v_{degradation} = k_{degradation} \cdot Vli \cdot (1 + \frac{cho}{kr}) \tag{14}$$

The reactions depend on the parameter kr, which regulates the impact of the cholesterol concentration on the synthesis (inhibition) or degradation (activation). The rate constants $k_{synthesis}$ and $k_{degradation}$ determine the rate of protein synthesis and protein degradation, respectively.

The model assumption was made that the cholesterol concentration influences the synthesis and degradation of all of the three proteins in the same manner and identical parameter values were used for all three proteins.

HMG-CoA synthase The cholesterol biosynthesis is the central part of the model. The starting point is the synthesis of HMG-CoA from three molecules acetyl-CoA via the HMG-CoA synthase. The reaction was modelled as a first order kinetic with two regulatory mechanisms (equation 15).

$$v_{HMGCOASYNTHASE} = \frac{K_{cat} \cdot hmgcoasynthase \cdot acoa \cdot V_{li}}{1 + \frac{hmgcoa}{K_i}} \tag{15}$$

The reaction kinetic includes a product inhibition from HMG-CoA and a dependence on the protein amount of HMG-CoA synthase. The amount of hmgcoasynthase linearly influences the maximum velocity of the reaction, determined by the term $Kcat \cdot hmgcoasynthase$. The product inhibition was implemented to avoid HMG-CoA over-accumulation, when the following step of the conversion of HMG-CoA is inhibited. The strength of the product inhibition is determined by the parameter $HMGCOASYNTHASE\_K_i$. Acetyl-CoA was assumed to be constant, to maintain a steady pool of precursors.

HMG-CoA reductase The subsequent steps in the cholesterol biosynthesis were lumped in a single reaction step, which directly catalyzes cholesterol from HMG-CoA. One molecule of HMG-CoA is converted to 6-isopentylpyrophosphate and six of these molecules are used to form one molecule cholesterol via multiple steps. This results in an overall stoichiometry of six molecules HMG-CoA to one molecule cholesterol for the model.

HMG-CoA reductase was modelled as irreversible Michaelis-Menten kinetics with additional regulatory terms (equation 16).

$$v_{HMGCOAREDUCTASE} = \frac{K_{cat} \cdot hmgcoareductase \cdot hmgcoa \cdot V_{li}}{K_m(1 + \frac{simacid + f_{activity} \cdot simmets}{K_i}) + hmgcoa} \tag{16}$$

The velocity of this reaction depends on the parameter K_m and the inhibition constant K_i. Additionally the concentration of the protein amount hmgcoareductase linearly determines the maximum velocity, with the term $K_{cat} \cdot hmgcoareductase$.

The main effect of simvastatin is the inhibition of HMG-CoA reductase via its metabolites. This inhibition does not only come from the main metabolite simvastatin acid but the simvastatin metabolites contribute to this inhibition as well. The parameter $f_{activity}$ determines the inhibitory activity of simvastatin metabolites which was assumed to be 0.5, i.e., simvastatin metabolites have 50% of the activity of simvastatin acid (in line with reported values). The active HMG-CoA reductase inhibitors as introduced in the introduction, are the sum of simvastatin acid and simvastatin metabolites weighted by $f_{activity}$.

The inhibition of HMG-CoA reductase by simvastatin is the main linking point between the pharmacokinetics model for simvastatin and the model for cholesterol biosynthesis and homeostasis. The inhibition constant K_i determines the affinity of active HMG-CoA reductase inhibitors for the enzyme HMG-CoA reductase.

Cholesterol rates Hepatic cholesterol can be exported into the plasma as VLDL-cholesterol, which is converted in the body to LDL-cholesterol. The main focus of this work was the description of LDL-cholesterol. Therefore, the process of packing cholesterol into VLDL particles and the subsequent conversion into LDL was lumped to a single reaction. The export and transformation to LDL-cholesterol were highly simplified to a reaction directly converting hepatic cholesterol to plasma LDL-cholesterol with an irreversible Michaelis-Menten kinetic.

$$v_{LDLCEXP} = V_{max} \cdot V_{li} \frac{cho_{li}}{K_m + cho_{li}} \tag{17}$$

The import of LDL-cholesterol from the plasma and its conversion back to hepatic cholesterol was in an analogue manner described by a simplified reaction with irreversible Michaelis-Menten equation.

$$v_{LDLCIMP} = K_{cat} \cdot V_{li} \cdot ldlreceptor \frac{ldlc_{ext}}{K_m + ldlc_{ext}} \tag{18}$$

Importantly, the maximum velocity of this concentration depends linearly on the concentration of the LDL receptor in the liver.

The excretion of cholesterol as bile acids or unchanged cholesterol in feces is lumped in a single reaction describing hepatic cholesterol loss. This reaction and the dietary uptake of cholesterol are modelled as zero order kinetic, i.e., as constant rate of loss and dietary uptake with rates in line with reported daily cholesterol loss and dietary uptake.

$$v_{dietaryuptake} = k_{dietaryuptake} \cdot V_{li} \tag{19}$$

$$v_{cholesterolloss} = k_{cholesterolloss} \cdot V_{li} \tag{20}$$

Lastly, a cholesterol utilization reaction on the whole body level was included, which consumes LDL-cholesterol (e.g the usage of cholesterol to build up

cell membranes). The kinetic of this reaction was assumed to follow Michaelis-Menten kinetic.

$$V_{ldlc-utilization} = V_{max} \cdot BW \frac{ldlc_{ext}}{K_m + ldlc_{ext}} \tag{21}$$

This equation depends on the parameter K_m and the LDL-cholesterol concentration in the venous blood $ldlc_{ext}$. The maximum velocity is determined by the parameter V_{max}. The reaction scales with the bodyweight BW.

Summary In summary, a detailed physiological-based pharmacokinetics model of simvastatin and its metabolites was developed including tissue models of the intestine/gut, the liver and the kidneys. Furthermore, a simplified model of hepatic cholesterol homeostasis was developed including the key steps of cholesterol biosynthesis in the liver and rates relevant for cholesterol homeostasis (loss, dietary uptake, utilization). Transformation of hepatic cholesterol and the utilization as LDL-cholesterol was added. The main coupling point between the pharmacokinetics model of simvastatin and cholesterol biosynthesis is the inhibition of HMG-CoA reductase by active simvastatin inhibitors.

3.3 Model parametrization

Next step was parametrization of the model. A subset of parameters for the simvastatin and cholesterol model could be obtained from the literature. A literature search for kinetic parameters and reference concentrations for simvastatin, its metabolites, cholesterol and its precursors involved was performed. The results from this literature research are presented in the supplement in section 6.6.

3.3.1 Parametrization of simvastatin model

Literature values The hepatic uptake of simvastatin and its metabolites are mediated by the OATP1B1 transporter and modelled via a reversible Michaelis-Menten kinetics. Based on research the K_m-values for the transporters $LI\_\_SIMVAIMP$ and $LI\_\_SIMACIDIMP$ were set to 9.7 μM for simvastatin and 3.6 μM for simvastatin acid [7], respectively. The hepatic uptake of simvastatin metabolites was assumed to be similar due to the structural similarity of the molecules. The K_m-value $LI\_\_SIMMETSIMP$ was set to the mean of simvastatin and simvastatin acid, 6.7 μM. Identical K_m-values for the transport reactions in the intestine and the liver were assumed for simvastatin, simvastatin acid and simvastatin metabolites.

The conversion of simvastatin to simvastatin acid is mediated by esterases in the liver. A variety of K_m, K_i and IC50-values for esterases with simvastatin or simvastatin acid as substrates and inhibitors are reported in the literature. The reported K_i-value of 0.8 μM in human liver microsomes for the esterase CES1A1 in homo sapiens [19] was used as K_m for the esterase reaction.

The reaction of simvastatin and simvastatin acid to simvastatin metabolites in the liver and intestine are mediated by cytochrome P450 (CYP) enzymes. The CYP3A4 isoform is the major isoform involved in this reaction and expressed in liver and intestine. The assumption was made that the K_m-value is identical for the conversion of both substances in both tissues (the identical

isoform of CYP3A4 is expressed in liver and intestine). Literature research obtained K_m-values for the conversion of simvastatin acid to simvastatin metabolites specifically for CYP3A4-conversion. These values were 21.6 and 29 μM in three different assays [78]. Other assays examined the K_m-values by the conversion of simvastatin and simvastatin acid by human liver microsomes. These values were in the range of 20.9 - 36.2 μM for simvastatin [77] and 47 - 76 μM for simvastatin acid conversion [78]. The $CYP3A4\_Km$ value for the CYP3A4 conversion was set to 25 μM. The conversion of simvastatin and simvastatin metabolites via CYP3A4 was assumed to follow identical kinetics.

Parameter fitting The remaining parameters of the simvastatin model were determined using parameter fitting (sec. 2.4).

In total 16 model parameters were fitted for the simvastatin model based on simvastatin, simvastatin acid, total and active inhibitors timecourses and timecourses of the sum of simvastatin and simvastatin acid. The parameters accounted for kinetics in the absorption, distribution, elimination and in the import and export to the liver and intestine. Additionally parameters for the biochemical reactions mediated by the esterases and CYP3A4-enzymes were fitted.

The following 20 studies after single oral dose of simvastatin in healthy subjects with in total 40 timecourses were used for parameter fitting fitting. The respective reported substances are listed for the studies.

- **Backman2000** [2]: simvastatin, simvastatin acid
- **Chung2006** [11] :simvastatin
- **Gehin2015** [21]: simvastatin, simvastatin acid
- **Jacobson2004** [37]: simvastatin, simvastatin acid
- **Jiang2017** [38]: simvastatin, simvastatin acid
- **Kantola1998** [41]: simvastatin, simvastatin acid
- **Kim2019** [44] : simvastatin, simvastatin acid
- **Kyrklund2000** [49]: simvastatin, simvastatin acid
- **Lilja1998** [53] : simvastatin, simvastatin acid, active HMG-CoA reductase inhibitors, active HMG-CoA reductase inhibitors
- **Lilja2000** [54]: simvastatin, simvastatin acid
- **Lilja2004** [55] : simvastatin, simvastatin acid
- **Lohitnavy2004** [57]: simvastatin, simvastatin (two simvastatin formulations)
- **Marino2000** [62] : simvastatin and simvastatin acid (as sum)
- **Mousa2000** [68]: simvastatin
- **Neuvonen1998** [70]: simvastatin and simvastatin acid (as sum), total HMG-CoA reductase inhibitors
- **Pasanen2006** [74] : simvastatin and simvastatin acid
- **Pentikainen1992** [75]: active HMG-CoA reductase inhibitors, total HMG-CoA reductase inhibitors
- **TubicGrozdanis2008** [86]: simvastatin, simvastatin acid (two simvastatin formulations)
- **Ucar2004** [88]: simvastatin, simvastatin acid
- **Zhou2013** [96]: simvastatin

Importantly, only simulation experiments and studies for single dose simvastatin application were used in model fitting. All studies with multiple dose simvastatin application were used as validation data. These include 6 studies with in total 11 timecourses:

- **Bergman2004** [3]: simvastatin, simvastatin acid, active HMG-CoA reductase inhibitors, total HMG-CoA reductase inhibitors
- **Hsyu2001** [32]: active HMG-CoA reductase inhibitors
- **Jacobson2004** [37]: simvastatin
- **Simard2001** [81]: simvastatin, simvastatin acid
- **Zhi2003** [95]: simvastatin and simvastatin acid
- **Ziviani2001** [97]: simvastatin and simvastatin acid (as a sum)

Some studies showed large deviation from the remaining studies and were excluded from the parameter fitting and subsequent analysis. These are mainly studies in non-healthy subjects and or subjects with a specific ethnicity. The excluded studies with respective reasons for exclusion are:

- **Cheng1994** [8]: Not healthy subjects (subjects underwent cholecystectomy)
- **Harvey2018** [26]: Not healthy subjects. In addition only geometric mean data were reported.
- **OBrien2003** [72]: Not healthy subjects (chronic myeloid leukaemia)
- **Najib2003** [69]: Simvastatin and simvastatin showed large deviation; possibly due to ethnicity and genetic variants (Arabian ethnicity)
- **Sugimoto2001** [85]: Simvastatin and simvastatin showed large deviation; possibly due to ethnicity and genetic variants (Japanese ethnicity)
- **Lohitnavy2004** [57]: Not clearly reported what was measured in the study.
- **Prueksaritanont2002** [79]: Active and total HMG-CoA reductase inhibitors showed large deviation (reason unclear, probably reporting issue)

In total 23 timecourses for simvastatin (19 single dose and 4 multiple dose as validation data), 17 timecourses for simvastatin acid (14 single dose and 3 multiple dose as validation data), 3 timecourses for simvastatin and simvastatin acid as a sum (2 single dose and 1 multiple dose as validation data), 4 timecourses for active HMG-CoA reductase inhibitors (2 single dose and 2 multiple dose as validation data) and 4 timecourses for total HMG-CoA reductase inhibitors (3 single dose and 1 multiple dose as validation data) were used in the fitting procedure and subsequent model evaluation.

The resulting optimal parameters are listed in Tab. 6. In the following we refer to the model with optimal parameters as 'reference model'. If not otherwise stated all simulations were performed with the reference model. If additional parameters were adjusted these are mentioned in the respective simulations and figures.

Table 6: Parameters included in the parameter fitting and their optimal values. It lists the parameter name as represented in the model, a short description of the parameter, the optimal value after fitting, and the unit. The shown values are parameter fitting results rounded to the fourth digit.

Parameter	Description	Value	Unit
Ka_dis_simva	SV dissolution rate	1.0289	$\frac{1}{hour}$
Ka_abs_simva	SV absorption rate	0.4282	$\frac{1}{hour}$
$ftissue\_simva$	SV distribution rate into tissues	0.2014	$\frac{l}{min}$
$ftissue\_simacid$	SVA distribution rate into tissues	0.01484	$\frac{l}{min}$
$LI\_\_SIMVAIMP\_Vmax$	v_{max} of SV transport in the liver	47.7479	$\frac{mmole}{min \cdot l}$
$LI\_SIMACIDIMP\_Vmax$	v_{max} of SVA transport in the liver	4.2756E-3	$\frac{mmole}{min \cdot l}$
$LI\_SIMMETSIMP\_Vmax$	v_{max} of SVM transport in the liver	0.06248	$\frac{mmole}{min \cdot l}$
$LI\_ESTERASE\_Vmax$	v_{max} of the reaction of SV to SVA in the liver	0.1176E-3	$\frac{mmole}{min \cdot l}$
LI_CYP3A4_Vmax	v_{max} of the reaction of SV and SVA to SVM in the liver	3.0882E-3	$\frac{mmole}{min \cdot l}$
$GU\_SIMVAEXP\_Vmax$	v_{max} of SV transport in the intestine	184.4580	$\frac{mmole}{min \cdot l}$
$GU\_SIMACIDEXP\_Vmax$	v_{max} of SVA transport in the intestine	1.6099e-06	$\frac{mmole}{min \cdot l}$
$GU\_SIMMETSEXP\_Vmax$	v_{max} of SVM transport in the intestine	0.3216	$\frac{mmole}{min \cdot l}$
GU_CYP3A4_Vmax	v_{max} of the reaction of SV and SVA to SVM in the intestine	0.3972	$\frac{mmole}{min \cdot l}$
$LI\_\_BILEINFLOW\_simmets\_k$	k of the SVM import into the bile	247.2279	$\frac{1}{min}$
$SIMMETSCL\_k$	v_{max} of the SVM clearance into the feces	27.8647	$\frac{1}{min}$
$KI\_\_SIMMETSCL\_f$	factor determining the fraction of the urinary clearance from the fecal clearance for SVM in the kidney	0.0580	$dimensionless$

3.3.2 Parametrization of cholesterol model

For the parametrization of the cholesterol model, initial/reference concentrations for hepatic cholesterol, HMG-CoA and acetyl-CoA, Michaelis-Menten constants and inhibition constants for the enzymes and rates for cholesterol balance were required.

Concentrations and parameters for biosynthesis Literature research obtained reference concentrations for hepatic acetyl-CoA of 0.043 ± 0.031 nmol/g wet weight in human liver microsomes [13]. Another study reported a range of 0.028 to 0.081 nmol/g wet weight in liver in rats [59]. These values were converted into μmol/l assuming the wet weight of liver tissue of 1 mg/ml. The reported concentration in human liver microsomes, supported by the rat studies, was used as the initial liver concentration for hepatic acetyl-CoA. It was set

to 0.043 mM.

For the hepatic concentration of HMG-CoA the literature research obtained a reference value of 0.022 ± 0.027 nmol/g wet weight in human liver microsomes [13]. As described above the value was converted to μmol/l and the model concentration of hepatic HMG-CoA was set to 0.022 mM. The reaction of the HMG-CoA synthase in the model (acetyl-CoA to HMG-CoA) includes a product inhibition. The corresponding Ki-value of the product inhibition LI__HMGCOASYNTHASE_Ki_hmgcoa was estimated using the HMG-CoA reference concentration and set to 0.022 mM, as well.

Studies reported a total free cholesterol concentration range of 5.8 - 160 nmol/g in the liver of mice [10], 8.17 nmol/g in the liver of rats, and 10 nmol/g in liver mitochondria of mice [83]. These values were converted into μmol/l as mentioned above. Based on these findings the initial hepatic cholesterol concentration was set to 0.02 mM. The export reaction of cholesterol uses a Michaelis-Menten kinetics. Using the reference concentration of hepatic cholesterol the Km-value of this kinetic LDLCEXP_Km was assumed to be in normal concentration range and set to 0.02 mM.

The kinetic of the HMG-CoA reductase was parametrized based on literature research as well. Studies report a Km-value for the conversion of HMG-CoA of 4 μM [36] and an inhibition constant of simvastatin on the HMG-CoA reductase of 0.12 nM [16]. These values were set in the model for the parameters LI__HMGCOAREDUCTASE_Km and LI__HMGCOAREDUCTASE_Ki_simacid, respectively.

Hepatic cholesterol homeostasis To model hepatic cholesterol homeostasis all liver reactions and rates needed to be balanced in the reference state: This means without the addition of simvastatin acid, with an initial hepatic cholesterol concentration of 0.02 mM and a plasma LDL-cholesterol concentration of 3 mM (which is the cut-off value for hypercholesterolemia, sec. 1.1.4). No net plasma LDL-cholesterol change should occur under these conditions.

In line with literature data dietary uptake and synthesis were set to 1 g/day each, the cholesterol loss was set to 1.2 g/day and the net export to the plasma to 0.8 g/day. The net export is the difference between plasma export and import. These rates create a balanced homeostasis, with 2 g/day of cholesterol being lost or exported and 2 g/day being synthesized or taken up by the liver.

All in all there are five reactions, that directly influence hepatic cholesterol levels. In the parametrization strategy the $vmax$, $Kcat$ or K values of these reactions were scaled with a reference parameter v_cho_reference set to a net cholesterol turnover of 1 g/day.

The dietary uptake and cholesterol loss, which use zero order reactions could be directly set to the required rates. The parameters LI__cho_loss_k and LI__cho_diet_k were set to 1.2 · v_cho_reference and 1.0 · v_cho_reference, to set the loss and dietary uptake strictly on 1.2 and 1.0 g/day, respectively.

To create an net export of 0.8 g/day, the $Kcat$-values of the import and export were set to 2.0 · v_cho_reference and 2.8 · v_cho_reference respectively, which sums to a net export of 0.8 g/day. The values were multiplied by two, due to the use of Michaelis-Menten kinetics (the reference concentrations are exactly the K_m of the reactions, resulting in a flux of 0.5 maximal rate at reference concentrations).

The $Kcat$-value HMGCOAREDUCTASE_Kcat of the reaction HMG-CoA to cholesterol, mediated by the HMG-CoA reductase is multiplied with $1.0 \cdot$ v_cho_reference to set the synthesis in the reference-state to 1 g/day.

The $Kcat$-value HMGCOASYNTHASE_Kcat of the reaction from acetyl-CoA to HMG-CoA, mediated by the HMG-CoA synthase was scaled with $350 \cdot$ v_cho_reference. Simply to be much higher than the HMG-CoA reductase to maintain a sufficient pool of HMG-CoA for cholesterol synthesis.

If no simvastatin acid is present in the liver, the synthesis is not inhibited and the cholesterol concentration does not vary. Therefore the protein amount for the ldl-receptor, hmgcoasynthase and -reductase were set to 1 mM at the reference point, and do not affect the respective reactions in steady state conditions.

Parametrization of plasma LDL-cholesterol Additionally to the liver model, a reaction accounting for the utilization of LDL-cholesterol is implemented in the whole-body. In the steady-state plasma LDL-cholesterol needs to be constant. The rates of the hepatic uptake and export of cholesterol produce a net export of LDL-cholesterol into the plasma. To maintain constant plasma LDL-cholesterol levels, the utilization needs to be balanced to the net export of 0.8 g/day.

The Michaelis-Menten constant K_m of utilization reaction is set to the reference-value of LDL-cholesterol of 3 mM and the maximal velocity v_{max} is set to $0.8 \cdot$v_cho_reference, to compensate the net export.

At initial plasma LDL-cholesterol levels of 3 mM the cholesterol fluxes are nicely balanced. However, it is not robust against changes in the initial LDL-cholesterol. A main application of the model was to evaluate effects of simvastatin therapy on different LDL-cholesterol levels. Simply setting the initial concentration to increased plasma LDL-cholesterol values, results in unbalanced rates and the concentration is drifting away from the initial concentration towards the reference state of the model.

To achieve steady-state at various initial LDL-cholesterol levels a compensation factor was introduced to scale the parameter v_cho_reference. The above described factor of 0.8 for the utilization in the reference state can be understood as the value for this factor in those conditions.

Multiple timecourses without simvastatin administration and varying initial LDL-cholesterol levels were simulated with varied compensation factor. Afterwards, the factor, which lead to constant LDL-cholesterol concentrations for 3 weeks of simulation, were evaluated and plotted against the initial concentration.

A double exponential regression was performed for the factors which achieved steady state, resulting in an equation, with which the correct compensation factor based on a given initial concentration could be calculated. This allowed to set any initial concentration of plasma LDL-cholesterol with balanced utilization and net export of the liver. In short, changing the utilization relative to the net export of cholesterol allows to achieve various steady states of plasma LDL-cholesterol.

3.4 Simvastatin pharmacokinetics

3.4.1 Time courses of simvastatin and metabolites

A main result of the work is a validated pharmacokinetic model of simvastatin. The model is able to predict timecourses for simvastatin, simvastatin acid, active and total HMG-CoA reductase inhibitors after single or multiple interventions of various simvastatin doses.

In total timecourses from 25 publications with 20 experiments with single dose and 6 experiments with multiple dose administrations of simvastatin were simulated.

Representative simulation experiments for single and multiple dose experiments are shown in Fig. 14 and in Fig. 15. The remaining simulation experiments are provided in the supplement (sec. 6.1 and sec. 6.2).

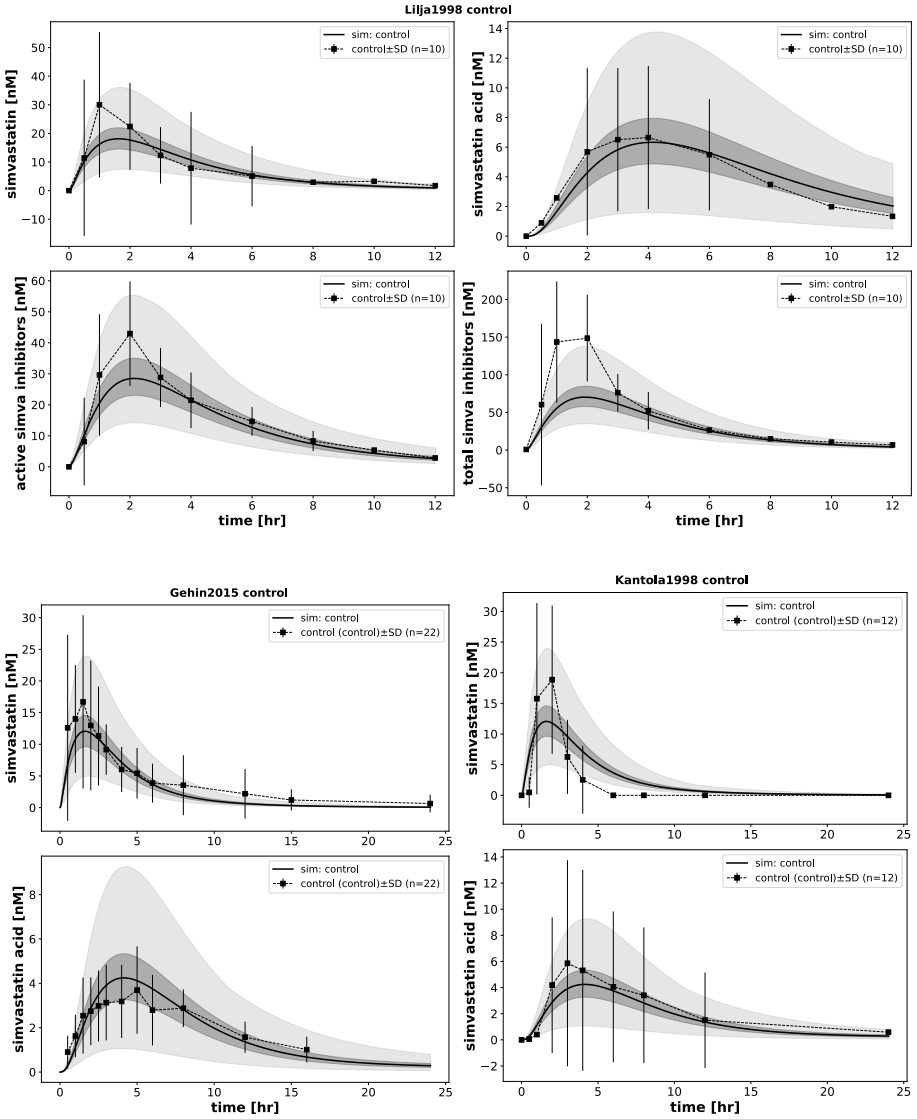


Figure 14: Representative experiments for single dose simvastatin. The depicted data are from the publications Lilja1998 [53], Gehin2015 [21] and Kantola1998 [41] including timecourses for simvastatin, simvastatin acid, active and total HMG-CoA reductase inhibitors with SD values and counts. The simulation time curves and the sensitivity are plotted for each timecourse.

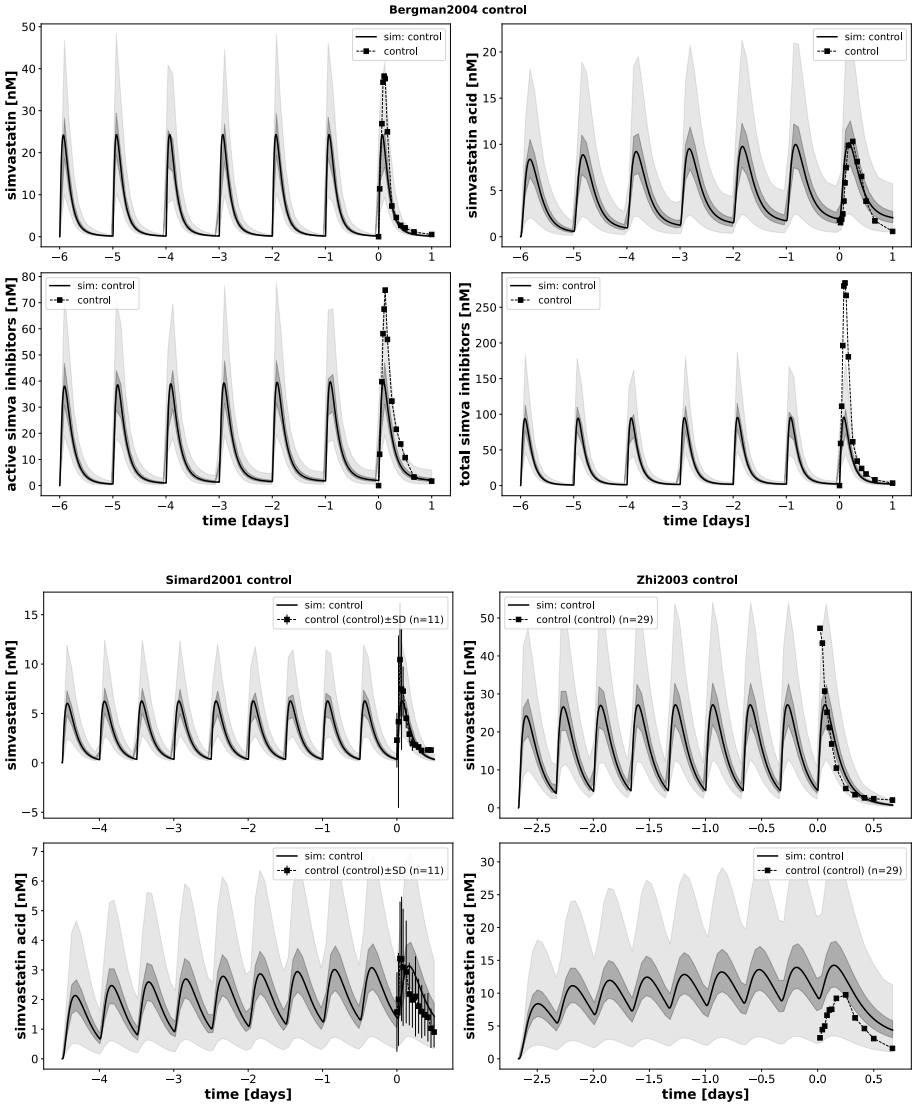


Figure 15: Representative experiments for multiple dose simvastatin. The data are from the publications Bergman2004 [3], Simard2001 [81] and Zhi2003 [95] including data for simvastatin, simvastatin acid, active and total HMG-CoA reductase inhibitors with SD values and counts. Simvastatin was given orally daily. The simulation time curves and the sensitivity are plotted for each time-course.

Overall, the model predicted a wide range of studies from various sources and under different doses very well. The validation timecourses under various multi-dose strategies were predicted well too.

3.4.2 Pharmacokinetic parameters of simvastatin and simvastatin acid

In a next step the pharmacokinetic model was validated using pharmacokinetics parameters. Simvastatin pharmacokinetic parameters were calculated on the predicted time courses and the dose-dependency of each parameter was evaluated. The data from the meta-analysis was used to compare and validate model predictions. The results of $AUC_{0-\infty}$, C_{max} , t_{max} and volume of distribution V_d are depicted in Fig. 16. The remaining parameters are provided in the supplement (sec. 6.3).

The predicted dose-dependency of the pharmacokinetic parameters is in very good agreement with the experimental data for simvastatin and simvastatin acid. Especially the dose-dependency of AUC_{0-end} and C_{max} is nicely reproduced by the model. All predicted pharmacokinetics parameters are within the range of the validation data.

Some outliers exist in the dataset. Many are from the study TubicGrozdanis2008 [86]. They used slow-release capsules for simvastatin, which altered the pharmacokinetics of simvastatin. The reference model can not predict those types of administration. A refit of the absorption and dissolution parameters would be required with the respective data. Furthermore, for simvastatin acid $AUC_{0-\infty}$ and C_{max} the study Pasanen2006 [74] is a clear outlier. Here, different genotypes for the OATP1B1 transport, were analyzed resulting in altered pharmacokinetics. Again a refit of the model parameters with the respective subset of data would allow to describe these data.

The model prediction interval of the model based on uncertainty analysis gives only a rough estimated of model uncertainty around the reference state. To model the large variability in the data set realistic ranges of all parameters in the models would be required.

In summary, the pharmacokinetic model reproduces a wide range of clinical and experimental data from multiple studies. Importantly, not only time courses under single or multiple dose could be reproduced and predicted but also the dose-dependency of pharmacokinetic parameters of simvastatin and simvastatin acid.

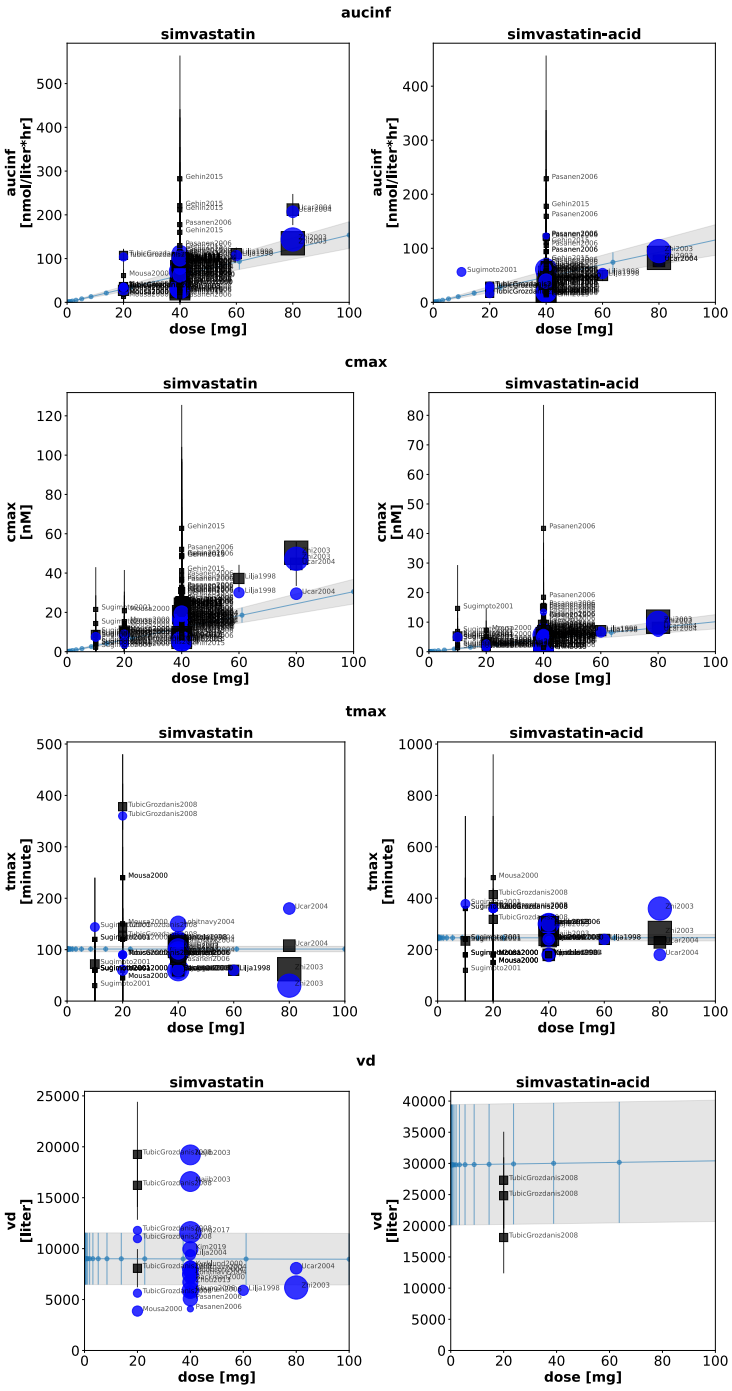
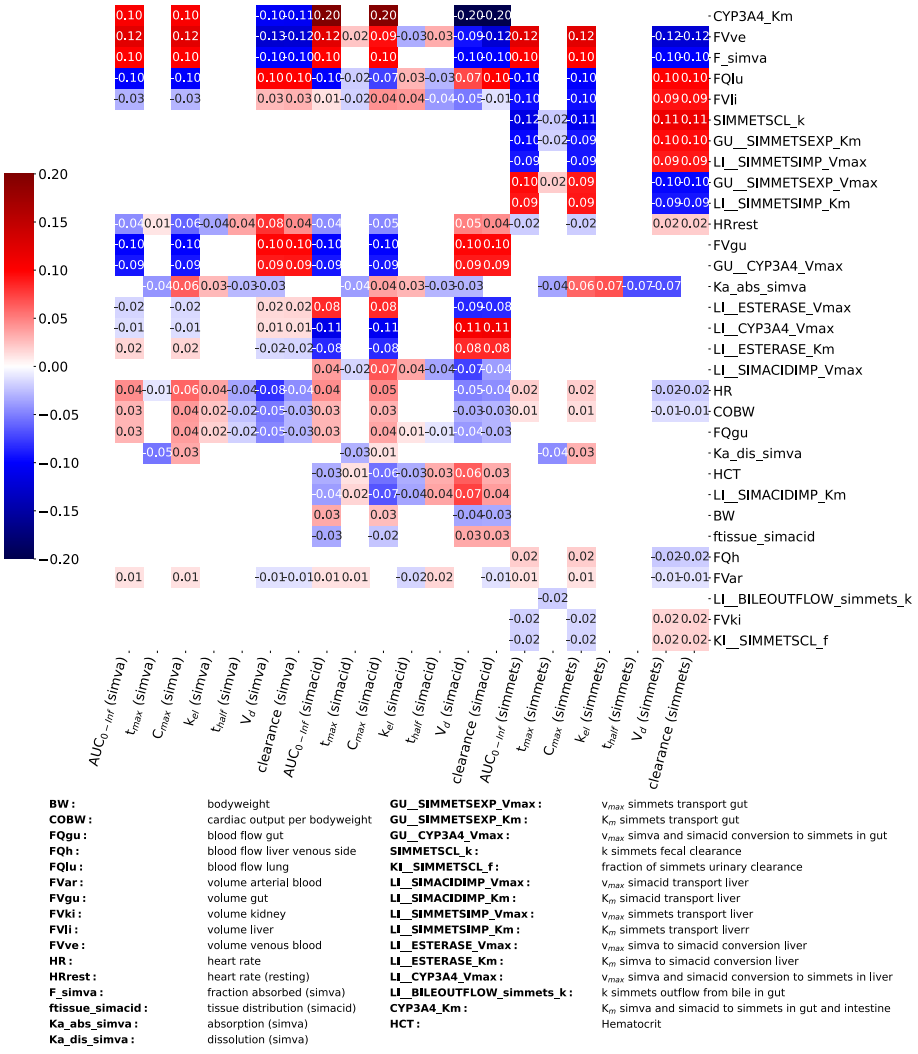


Figure 16: Dose-dependency of pharmacokinetic parameters $AUC_{0-\infty}$, C_{max} , t_{max} and V_d for simvastatin and simvastatin acid. Shown are the dose-dependencies in the range of simvastatin doses from 1 to 100 mg predicted by the simvastatin model. The uncertainty areas are plotted as the blue shaded areas. Additionally the data from the meta-analysis was added for comparison. with reported values in blue and values calculated from experimental timecourses in black. Data points are mean and SD.

3.4.3 Sensitivity analysis

To quantify the sensitivity of the pharmacokinetics of simvastatin, simvastatin acid, and simvastatin metabolites on the model-parameter a sensitivity analysis as described in section 2.6 was performed.



BW :	bodyweight	**GU__SIMMETSEXP_Vmax :**	v_{max} simmets transport gut
COBW :	cardiac output per bodyweight	**GU__SIMMETSEXP_Km :**	K_m simmets transport gut
FQgu :	blood flow gut	**GU__CYP3A4_Vmax :**	v_{max} simva and simacid conversion to simmets in gut
FQh :	blood flow liver venous side	**SIMMETSCL_k :**	k simmets fecal clearance
FQlu :	blood flow lung	**KI__SIMMETSCL_f :**	fraction of simmets urinary clearance
FVar :	volume arterial blood	**LI__SIMACIDIMP_Vmax :**	v_{max} simacid transport liver
FVgu :	volume gut	**LI__SIMACIDIMP_Km :**	K_m simacid transport liver
FVki :	volume kidney	**LI__SIMMETSIMP_Vmax :**	v_{max} simmets transport liver
FVli :	volume liver	**LI__SIMMETSIMP_Km :**	K_m simmets transport liverr
FVve :	volume venous blood	**LI__ESTERASE_Vmax :**	v_{max} simva to simacid conversion liver
HR :	heart rate	**LI__ESTERASE_Km :**	K_m simva to simacid conversion liver
HRrest :	heart rate (resting)	**LI__CYP3A4_Vmax :**	v_{max} simva and simacid conversion to simmets in liver
F_simva :	fraction absorbed (simva)	**LI__BILEOUTFLOW_simmets_k :**	k simmets outflow from bile in gut
ftissue_simacid :	tissue distribution (simacid)	**CYP3A4_Km :**	K_m simva and simacid to simmets in gut and intestine
Ka_abs_simva :	absorption (simva)	**HCT :**	Hematocrit
Ka_dis_simva :	dissolution (simva)		

Figure 17: Results of the simvastatin sensitivity analysis. On the horizontal axis pharmacokinetic parameters for simvastatin, simvastatin acid and simvastatin metabolites after a single oral dose of 10 mg simvastatin are given. On the vertical axis the changed parameters, which had an impact of at least 1% on the pharmacokinetic parameters are plotted. Red indicates pharmacokinetic parameters that increased and blue indicates parameters that decreased when the model parameter was increased. The change is given in percentage.

The model-parameter with the overall highest impact is $CYP3A4\_Km$. It

directly mediates the conversion of simvastatin to simvastatin acid and simvastatin metabolites. The $AUC_{0-\infty}$ and volume of distribution V_d of simvastatin acid and simvastatin metabolites are impacted the most. As expected parameters involved in the conversion of simvastatin to simvastatin acid are the central parameters for the simvastatin model.

Other model-parameters that had a high sensitivity are parameters for fractional tissue volumes such as for the venous blood volume FVve, the liver volume FVli and the gut volume FVgu. The respective tissue volumes are scaled using these volume factors, with kinetics in the tissue models being scaled by the volumes as well. As expected, changes in tissue volumes have a strong effect on pharmacokinetics parameters.

Absorption and excretion parameters like F_simva, Ka_dis_simva and Ka_abs_simva also have an influence on simvastatin pharmacokinetics. This is as expected that the absorption and clearance have a high impact on the model behavior.

Interestingly absorption and clearance parameters for the gut and liver transport especially for the species simvastatin metabolites $SIMMETSCL_k$, $GU\_\_SIMMETSEXP\_Km$, $GU\_\_SIMMETSEXP\_Vmax$, $LI\_\_SIMMETSIMP\_Vmax$ and $LI\_\_SIMMETSIMP\_Km$ are highly sensitive. Simvastatin metabolites seems to play the central role in the model. Parameters affecting its concentrations are highly sensitive.

In summary it can be observed that many pharmacokinetic parameters for simvastatin, simvastatin acid and simvastatin metabolites are sensitive to changes in model parameters. The enzyme conversion mediated by the parameter $CYP3A4\_Km$ seems to be the central parameter of the model, affecting pharmacokinetics the most. Besides metabolic parameters also many physiological parameters play an important role, such as tissue volumes or absorption rates. The analysis allows to connect physiological parameters in the whole-body model to the pharmacokinetics parameters of simvastatin.

3.5 Effects of simvastatin on cholesterol metabolism

After studying the simvastatin model in isolation, the coupled pharmacokinetic/pharmacodynamic model was examined.

3.5.1 Effects on hepatic cholesterol metabolism

In a first step we were interested on the changes in hepatic cholesterol metabolism and homeostasis due to simvastatin therapy. Therefore, the liver model was studied in isolation. An initial test simulation checked, whether cholesterol hepatic homeostasis and the effects of simvastatin application on cholesterol biosynthesis and regulation behaved as expected and are within the physiological range (Fig. 18).

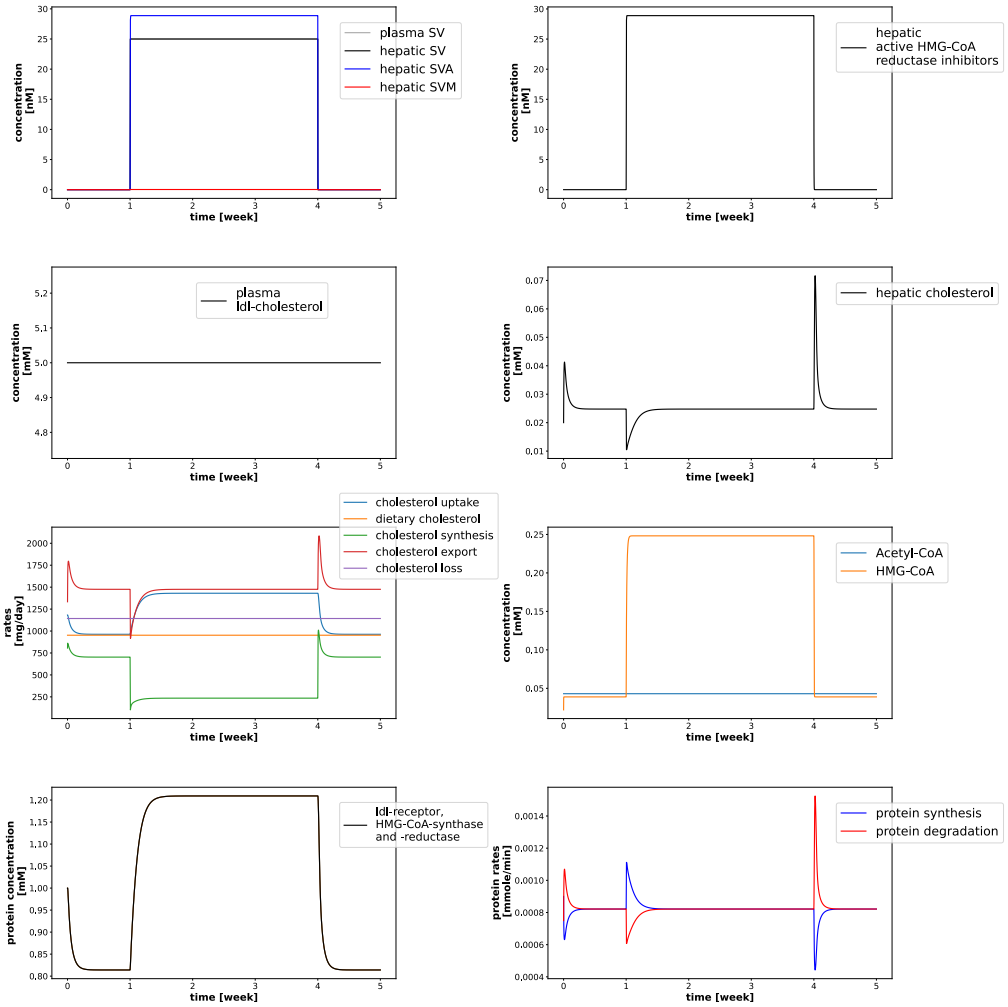


Figure 18: Simulation of the cholesterol model in the isolated liver in baseline and under simvastatin administration. The simulated experiment is split in three phases. Plasma LDL-cholesterol concentration is held constant. The first phase is without simvastatin and the model is simulated for one week with a plasma LDL-cholesterol concentration of 4.5 mM. In the second phase plasma simvastatin were held constant for 2 weeks at a concentration of 25 nM and withdrawn in the third phase to evaluate the behaviour of the model after simvastatin treatment. The figure presents plasma and hepatic concentration for simvastatin and its metabolites and cholesterol specific substances, like protein and precursor concentrations. Additionally rates of cholesterol biosynthesis, uptake, export, loss and dietary uptake are plotted, as well as protein synthesis and degradation rates.

First the liver model was simulated without simvastatin administration for one week. Throughout the whole experiment plasma LDL-cholesterol was held constant at 4.5 mM, which is a typical elevated plasma level. From weeks two

to four, a constant plasma simvastatin concentration of 25 nM was applied. Subsequently simvastatin was withdrawn and the experiment was simulated for an additional week.

In the first phase, steady state concentrations of cholesterol, cholesterol precursors, proteins and rates were reached. The hepatic dietary uptake of cholesterol is constant at 1000 mg/day and the loss constant at 1200 mg/day. These rates are constant throughout the whole experiment. The synthesis and the uptake and import are in the steady state at 750 mg/day and 1500 mg/day, respectively. Uptake and import are the same. Protein concentration go into a steady state, with balances rates of degradation and synthesis. It can be seen that the model reaches steady state concentration at a hepatic cholesterol level at slightly above 0.02 mM.

After simvastatin administration, hepatic levels of simvastatin, simvastatin acid, simvastatin metabolites and active HMG-CoA reductase inhibitors increase. The synthesis rate of cholesterol decreases immediately, resulting in decreased cholesterol levels and an accumulation of HMG-CoA (inhibition of HMG-CoA reductase by simvastatin). Additionally the uptake and export decrease. However after a few days, the protein synthesis rate increases and the degradation rate decreases. Overall hepatic protein amounts of LDL receptor and HMG-CoA synthase and reductase increase and reach a new plateau after one week. As a consequence the cholesterol uptake, export and synthesis increase. Still, the import and export are the dominating rates, while synthesis is not increasing as much, because of the very strong inhibition of active HMG-CoA reductase inhibitors.

The import and export rates produces a net import and hepatic cholesterol levels rise. The cholesterol steady state prior to simvastatin administration is reached again after one week. The slow counter-regulation by falling cholesterol levels via protein induction brings back hepatic cholesterol levels.

When simvastatin is withdrawn, the cholesterol overshoots due to the enforced biosynthesis, driven by HMG-CoA accumulation and high protein amount. The overshoot in cholesterol drives the export, which overshoots as well. The synthesis is not inhibited anymore and its rate increases, leading to decreased protein synthesis and increased protein degradation rates. The hepatic protein levels are lowered. As a consequence cholesterol uptake and export decrease again (due to reduced LDL receptors), and the steady state of the time before simvastatin administration is reached again.

The results suggest that the cholesterol model in the liver is working as expected. Cholesterol homeostasis can be explained as well as the regulative mechanisms after simvastatin administration work. An interesting dynamic takes places due to the combination of fast metabolic regulations and slower adaptations of protein levels due to changes in hepatic cholesterol.

3.5.2 Effects on plasma LDL-cholesterol

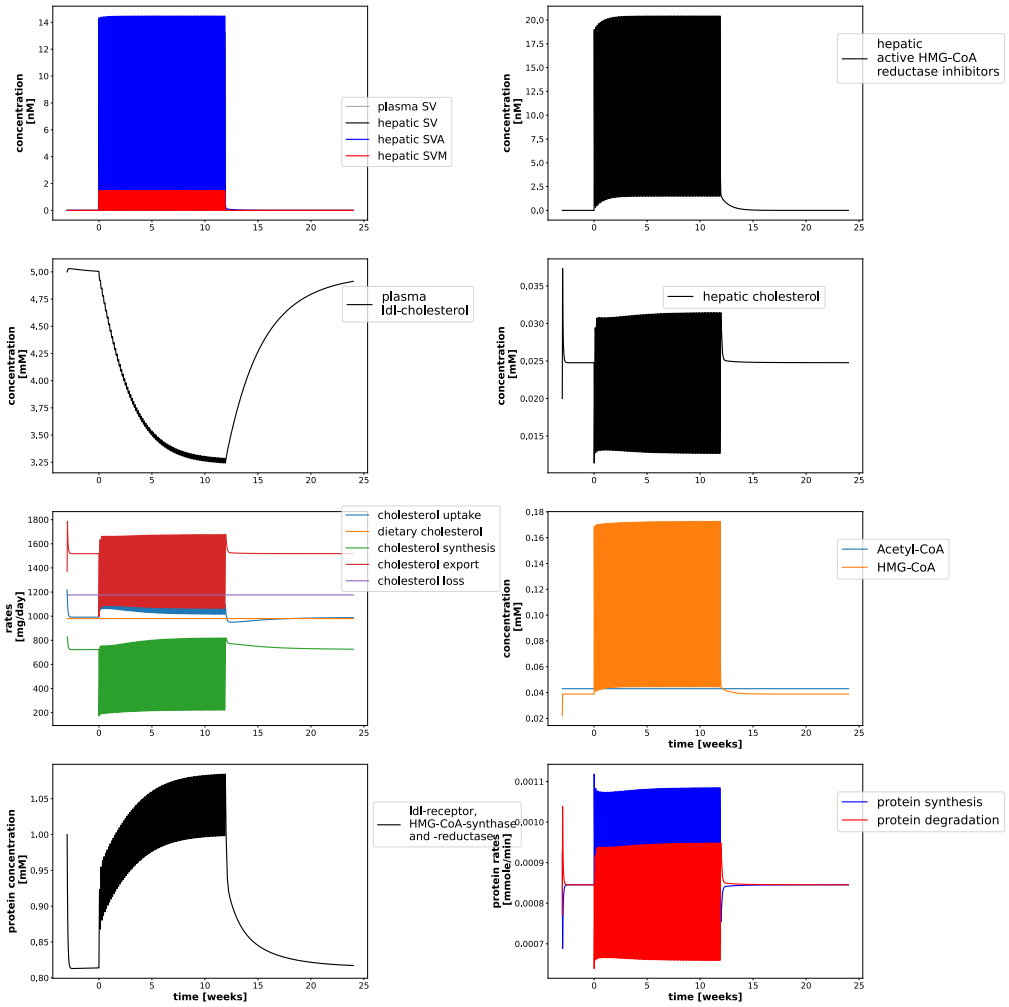


Figure 19: Hepatic cholesterol metabolism in the context of the whole-body model. The figure presents concentrations for plasma levels of simvastatin, active HMG-CoA reductase inhibitors and LDL-cholesterol and hepatic levels of simvastatin, simvastatin acid, simvastatin metabolites, cholesterol and protein concentrations. Rates of dietary cholesterol uptake, loss, hepatic export, import and of the cholesterol synthesis are shown. Initially the model was simulated for three weeks without simvastatin. Simvastatin intervention was performed on a daily basis for 12 weeks with 40 mg. After this period the model was simulated for another 12 weeks without simvastatin.

To test the model behavior in the context of the whole-body scale, a second simulation experiment was created. This experiment is important to see whether the observed effects in the liver model actually affect plasma LDL-cholesterol and lead to a decrease in the context of a dynamic whole-body model.

The experiment consists of three phases. Initially the model was simulated for three weeks without simvastatin administration to reach a steady state with constant plasma LDL-cholesterol and hepatic cholesterol levels. All other rates and concentrations prior to week zero are constant as well.

Daily simvastatin administration was performed for 12 weeks with a dose of 40 mg daily. Plasma simvastatin concentrations rise resulting in elevated concentration of hepatic simvastatin, simvastatin acid and simvastatin metabolites. Importantly, due to the fast half-life of simvastatin, plasma levels of simvastatin and its metabolites go almost back to baseline within 24 hr resulting in daily peaks. The concentration of active HMG-CoA reductase inhibitors rises, which leads to immediate inhibition of the cholesterol synthesis. All of the regulatory effects and rate changes, observed in the isolated liver model can be observed. Due to the daily dosing regime a spiking dynamics is overlayed on the slower changes occurring over weeks. Protein synthesis increases and degradation decreases, resulting in elevated protein concentration. These mediate an increased cholesterol uptake into the liver due to LDL-receptor expression, while the HMG-CoA reductase is still inhibited. The effect of the LDL-cholesterol lowering capacity of simvastatin can be seen. LDL-cholesterol levels decrease slowly, reaching a new plateau after 12 weeks.

After withdrawal of simvastatin, the simvastatin and metabolite levels decrease. This results in increased levels of cholesterol synthesis, leading to a net protein degradation and lowered protein levels. LDL-cholesterol increases again, reaching the initial levels after another 12 weeks after treatment cessation.

On a fast time scale cholesterol synthesis is inhibited due to competitive inhibition of HMG-CoA reductase by active HMG-CoA reductase inhibitors. The resulting fall in cholesterol activates transcriptional/ translational regulation and the protein system can elevate hepatic cholesterol again. It can be observed that the regulatory system acts on a time-scale of weeks for the LDL-cholesterol lowering effect. When simvastatin is withdrawn the model is capable of going back into the old steady state.

After testing the model isolated in the liver, the same mechanisms and behavior can be observed on a whole-body scale as well. In summary, we see that cholesterol homeostasis and the reported regulatory mechanisms after simvastatin application can be simulated after coupling the liver model to the whole body model as well. Interesting dynamic effects can be observed in the combination of a daily dosing scheme with slow acting changes in protein amounts.

3.5.3 Timecourses of cholesterol studies

In a next step we evaluated and validated model predictions with studies reporting changes in plasma LDL-cholesterol in simvastatin therapy.

The following studies with the reported substances were evaluated and plotted.

- **Jones1998a** [40]: total, HDL- and LDL-cholesterol, triglycerides
- **Kosoglou2002** [47]: total, HDL- and LDL-cholesterol, triglycerides
- **Ntanios1999** [71]: total, HDL- and LDL-cholesterol, triglycerides

- **Tuomilehto1995** [87]: total , HDL- and LDL-cholesterol, triglycerides
- **Walker1990** [94]: total and LDL-cholesterol

The study Loria1993 [58] was curated but excluded from the simulation experiments due to the very short therapy time (18 hr) and the resulting non-representative data for LDL-cholesterol change after simvastatin administration.

Representative simulation experiments are shown here with the remainders being provided in the supplement (sec. 6.4).

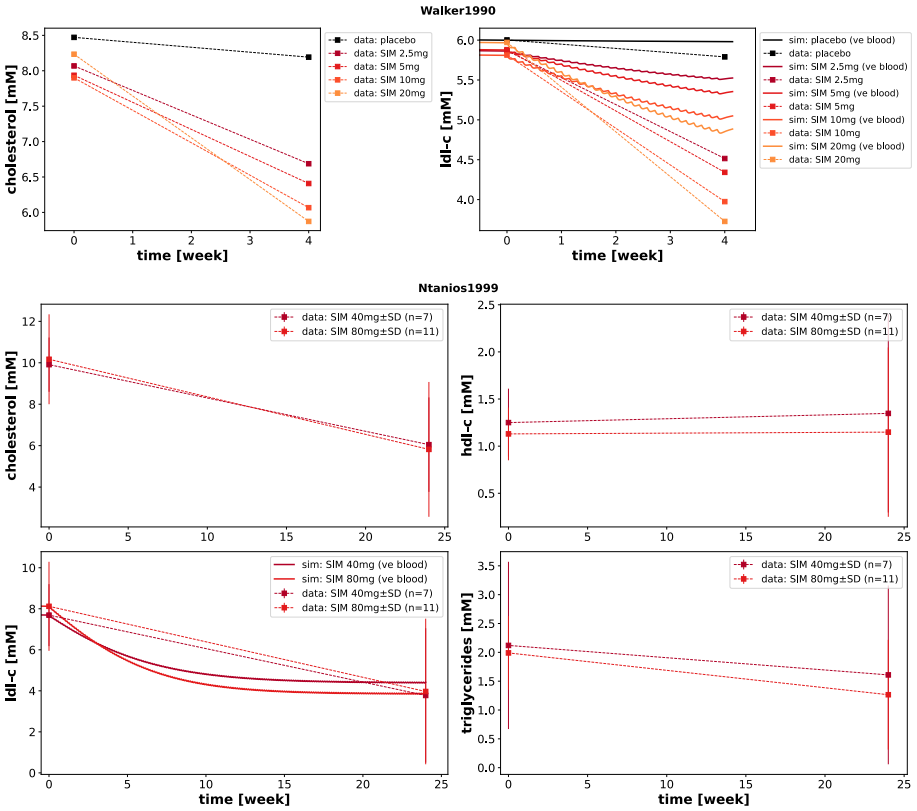


Figure 20: Representative simulation experiments after multiple dose applications of simvastatin. The changes of total cholesterol and LDL-cholesterol are plotted and simulation results were added for the LDL-cholesterol change. Examined doses of simvastatin are placebo, 2.5, 5, 10 and 20 mg.

The simulation experiments show that the decrease of LDL-cholesterol after simvastatin treatment can be predicted reasonably well. The actual decrease in therapy in the model predictions strongly depends on the dose and duration of simvastatin treatment.

3.5.4 Effects of dose and duration

Interval and dose experiments After validation of the cholesterol model with experimental data the effects of different duration, doses and dosing schemes of simvastatin therapy were analyzed systematically.

The first experiment was created to evaluate different dosing intervals and doses. Intervals of 12, 24 and 48 hr with different doses of placebo, 2.5, 5, 10, 20, 40 or 80 mg of simvastatin were simulated over a course of 25 weeks.

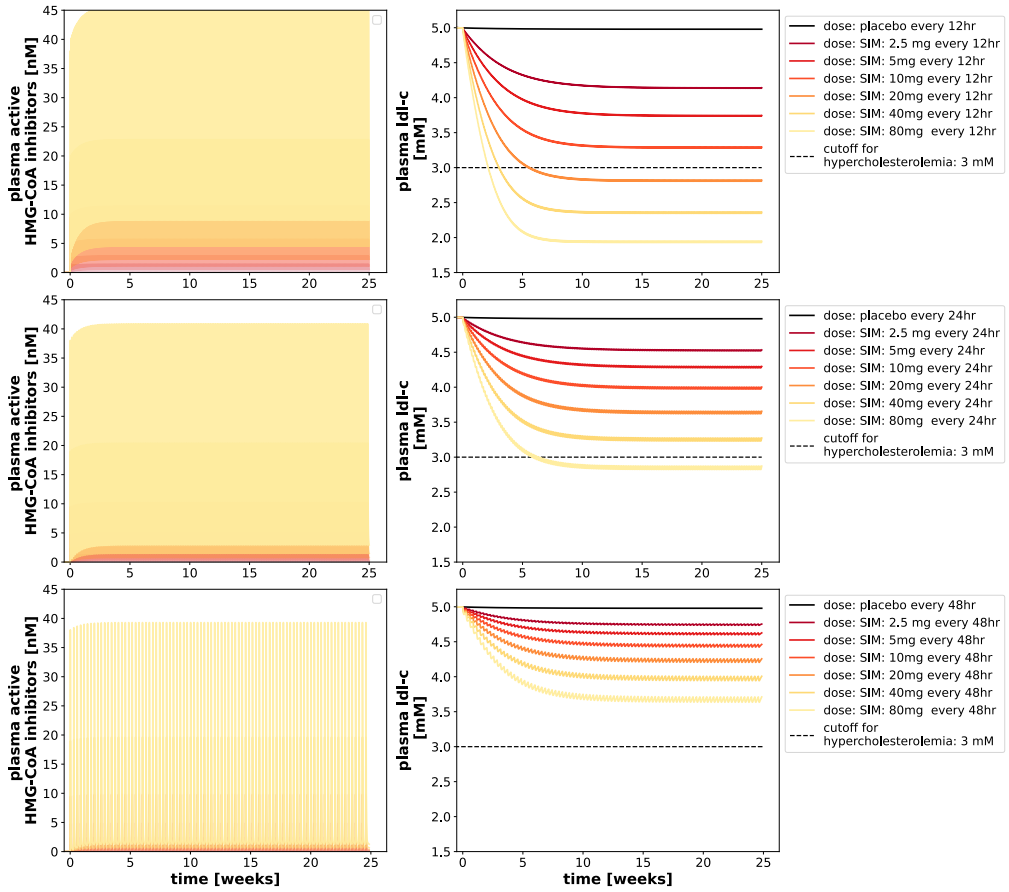


Figure 21: The dose-response curves of different simvastatin doses in varying conditions are shown over the time. Multiple interventions of either placebo, 2.5, 5, 10, 20, 40 or 80 mg of simvastatin were given in intervals of 12, 24 or 48 hours. Each treatment was simulated for 25 weeks. The plots one the left show the concentration of active HMG-CoA reductase inhibitors and the plots on the right side, the resulting plasma LDL-cholesterol concentration. Each simulation was initiated with a plasma LDL-cholesterol concentration of 5 mM and the cutoff value of 3 mM is plotted.

First of all it can be seen that the intervals have different effects on active HMG-CoA reductase inhibitors. At intervals of 48 hr, there are spikes in the plasma concentration, coming from the fast half-life of simvastatin. As the intervals are shorter, spikes are rare and there are steadier and higher plasma concentration throughout the experiment. These different concentrations of active HMG-CoA reductase inhibitors are highly affecting the plasma LDL-cholesterol change. Comparing the plots it can be seen, that doses of 20, 40,

80 mg for the 12 hr intervals are capable of decreasing LDL-cholesterol levels to the desired 3 mM. At intervals of 24 hr only 80 mg simvastatin doses and at intervals of 48 hr no therapy plan reaches the 3 mM cutoff. All therapies need as least 8-12 weeks to reach the maximum of LDL-cholesterol decrease in the model predictions.

The interval of treatment in combination with the actual dose plays a huge role in a therapy plan.

Dose-response experiments To study the duration of a treatment in detail a more detailed dose-response experiment was created. Simvastatin was administered daily for 1 to 25 weeks and the percentage change in LDL-cholesterol was studied.

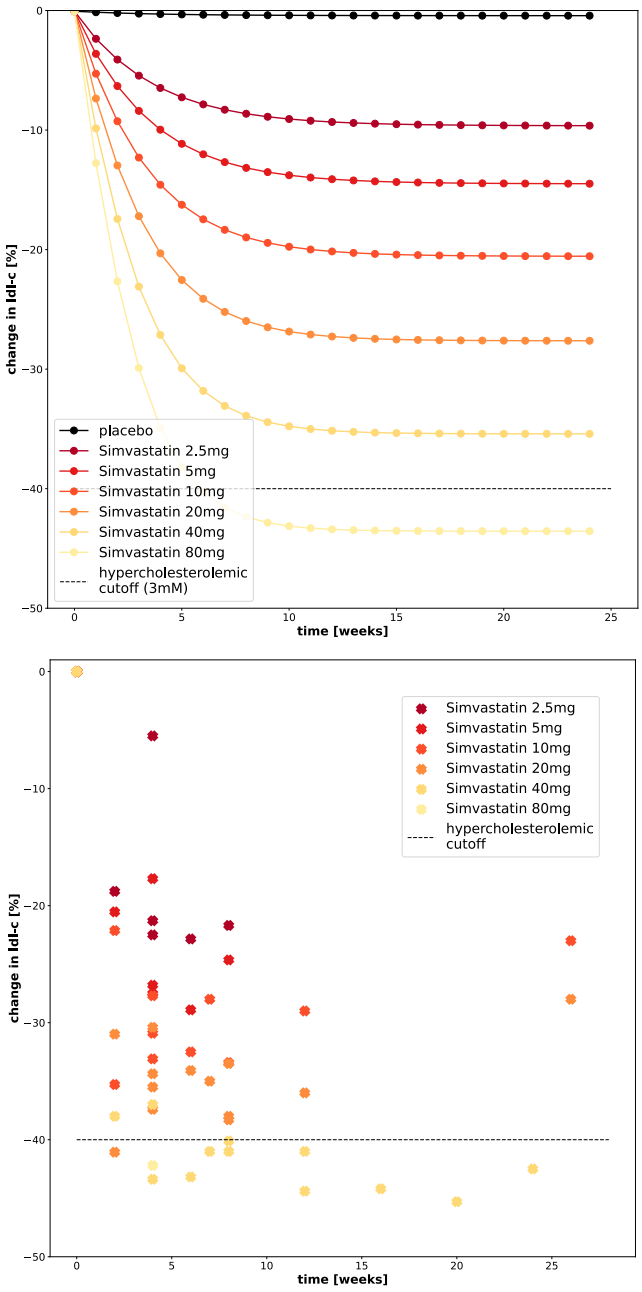


Figure 22: The upper figure shows the plasma LDL-cholesterol change in dependence of different simvastatin doses over a course of 25 weeks after daily oral simvastatin application of either placebo, 2.5, 5, 10, 20, 40 or 80 mg. At time 0 the initial concentration of plasma LDL-cholesterol for each timecourse was 5 mM. After each week the LDL-cholesterol decrease for each simvastatin dose was measured and plotted, beginning with week 1. The cutoff for normal plasma ldl-cholesterol levels at 3 mM, corresponding to a 40% decrease, is plotted as a reference value.
The lower figure shows the plasma LDL-cholesterol change in dependence of different simvastatin doses obtained from literature (sec. 6.5). The data points are the change in LDL-cholesterol after daily simvastatin treatment.

It can be seen that by increasing the simvastatin doses, the maximum plasma LDL-cholesterol decrease rises. As observed in Fig. 21 every treatment reaches a steady state and its maximum of change after 10-12 weeks.

To validate the simulated dose-response curves, the collected data for LDL-cholesterol changes after simvastatin therapy were plotted. The data confirms the model predictions that higher doses of simvastatin increase LDL-cholesterol changes. Overall a good agreement between the predicted LDL-cholesterol changes and observed changes by multiple studies can be seen. The data suggest that the maximal change is reached after shorter treatment duration in patients. Here the maximum change is reached after 4-8 weeks, implying that the model might not react fast enough. The timescale of the LDL-cholesterol decrease is too slow.

After evaluating the duration of treatment the dose-dependency of LDL-cholesterol under continuous therapy was evaluated. The changes of LDL-cholesterol after 25 weeks from Fig. 22 were plotted against the administered dose.

An important outcome is that plasma LDL-cholesterol change does not scale linearly with the simvastatin dose (in the model predictions as well as in the experimental data). It seems, as if there is a point from which increasing the simvastatin dose does not result in higher LDL-cholesterol changes. To examine the model behaviour for this in detail, higher doses would have been necessary. However, even 80 mg doses of simvastatin daily are not very commonly applied due to risk of serious side-effects.

The model predictions were compared to data from literature (sec. 6.5). Only data points reporting LDL-cholesterol changes after at least 12 weeks of daily simvastatin treatment were used. Qualitatively the model can describe the dose-dependence of the LDL-cholesterol change especially at doses from 5-20 mg. At higher doses the data predicts larger changes in LDL-cholesterol than the model. The analysis confirms, that the model is not sensitive enough. The data suggests that the model needs to be calibrated better, to be able to describe larger LDL-cholesterol decreases at simvastatin doses above 20 mg. Importantly, only minimal calibration of the cholesterol model was performed.

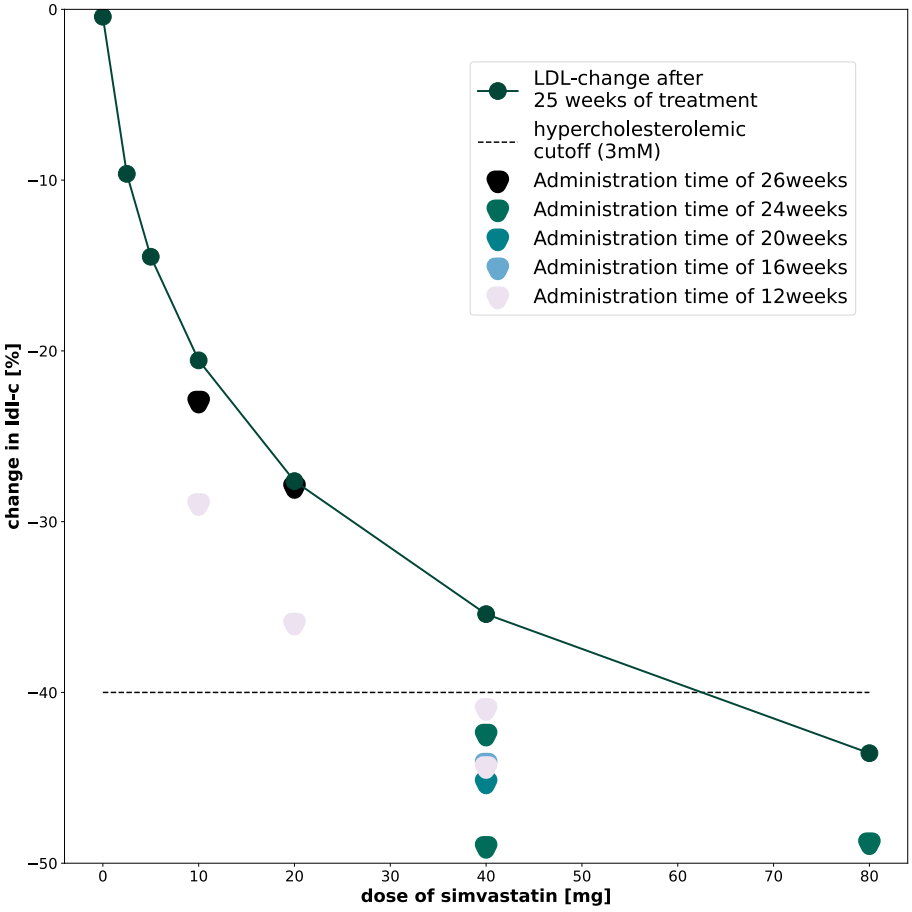


Figure 23: The endpoints of the LDL-cholesterol change after 25 weeks of treatment from Fig. 22 were plotted to evaluate the LDL-cholesterol change in dependence of the simvastatin dose. The experimental data for the LDL-cholesterol change was added (sec. 6.5). However only datapoints with at least 12 weeks of treatment were taken to be comparable to the 25 week duration of the the model prediction.

3.5.5 Sensitivity analysis

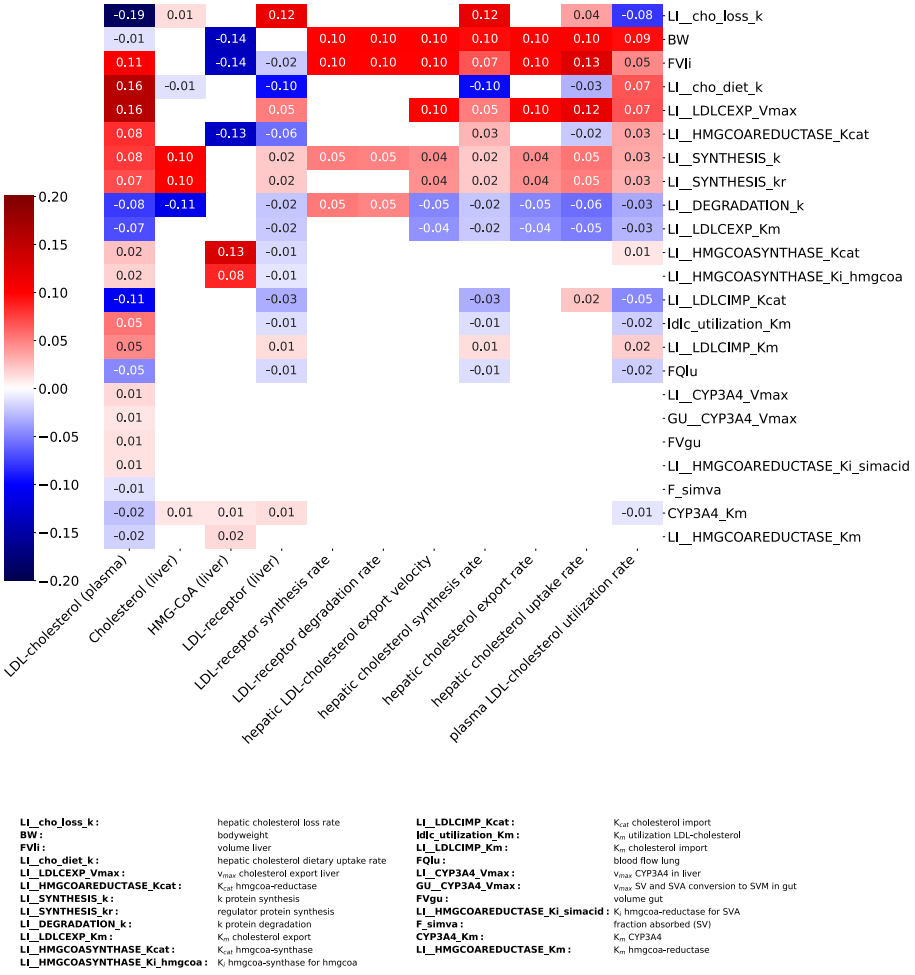


Figure 24: Results of the cholesterol sensitivity analysis. On the horizontal axis readouts for the cholesterol model, such as the mean cholesterol concentration over the last day of simulating are presented. The applied simulation was a 20 week treatment with 10 mg simvastatin daily. On the vertical axis the changed model parameters with the highest impact in pharmacokinetic parameters are plotted. The heatmap on the left shows the color mapping of the results. Red coloured results are parameters that were increased when the model parameter was changed and, blue coloured results represent parameters that decreased when the model parameter was changed. The change is given in percentage. SV, SVA and SVM stand for simvastatin, simvastatin acid and simvastatin metabolites, respectively.

Lastly, the effects of model parameters on cholesterol metabolism and plasma LDL-cholesterol levels were studied using sensitivity analysis.

The model-parameter $LI\_\_cho\_loss\_k$ had the highest sensitivity. It is the parameter with the highest impact on the plasma LDL-cholesterol. Still, plasma LDL-cholesterol is affected by every model-parameter change. Its level is the most sensitive from all cholesterol model readouts. Additionally the parameter bodyweight BW and the fractional liver volume $FVli$ are highly influencing the model readouts. Tissue volumes and bodyweight highly affect pharmacokinetics.

It can be seen that the protein regulation has major effects on the model. The parameters $LI\_\_SYNTHESIS\_k$, $LI\_\_SYNTHESIS\_kr$ and $LI\_\_DEGRADATION\_k$ are highly sensitive. They act as the main interface between inhibited cholesterol synthesis and LDL-cholesterol decrease. Changing them has an impact on almost every main model readout.

Highly affected model readouts are the plasma concentration of LDL-cholesterol, the hepatic cholesterol and ldlreceptor concentrations and the plasma LDL-cholesterol utilization rate. These are the main parameters involved in the decrease and the plasma concentration of LDL-cholesterol. Hepatic cholesterol is highly affected by protein concentrations and vise versa, making them sensitive readouts against model-parameter changes.

3.6 Summary

Data curation (sec. 3.1.1) provided a large set on simvastatin on cholesterol data which were analysed in the meta analysis (sec. 3.1.2). The data was used to develop a physiological based computational model of simvastatin was developed, describing absorption, distribution, metabolism and excretion of simvastatin and its metabolites (sec. 3.2.1). A model describing cholesterol pharmacodynamics was coupled to the simvastatin model, including the main reaction steps of cholesterol biosynthesis and includes cholesterol consumption, which accounts for usage in the body and excretion (sec. 3.2.2). Literature research and parameter fitting was performed to parametrize the simvastatin (sec. 3.3.1) and cholesterol model (sec. 3.3.2).

The model successfully predicted time courses of simvastatin, simvastatin acid and active and total inhibitors after single and multiple doses of simvastatin (sec. 3.4.1). Pharmacokinetic parameters and their dose dependency were successfully predicted and validated with data (sec. 3.4.2). Sensitivity analysis was performed to quantify which parameters are the most sensitive ones in the model and have the highest impact of simvastatin pharmacokinetics (sec. 3.4.3).

A model for cholesterol was developed, which is able to describe hepatic cholesterol homeostasis. It was possible to connect both model with an inhibition kinetic, that uses simvastatin acid as a competitive inhibitor of HMG-CoA-Reductase. This opened the possibility to describe hepatic cholesterol (sec. 3.5.1) and plasma LDL-cholesterol (sec. 3.5.2) decrease in simvastatin administration.

Changes in plasma LDL-cholesterol in various studies in simvastatin therapy were simulated and the effects of different dosing intervals, duration and doses of simvastatin were studied (sec. 3.5.4). The model was able to predict LDL-cholesterol change after simvastatin treatment. The sensitivity analysis gave insight into the most sensitive parameters of the cholesterol model and their impact on LDL-cholesterol change after simvastatin treatment (sec. 3.5.5).

4 Discussion

Data Within this project a high quality data collection for simvastatin pharmacokinetics and pharmacodynamics after single and multiple simvastatin doses was established. The data base includes timecourses and pharmacokinetic data for simvastatin, simvastatin acid, active and total HMG-CoA reductase inhibitors. An important part of the data-set are long-term studies reporting the effects of simvastatin therapy on total cholesterol, HDL-, LDL-cholesterol and triglycerides. Despite curating more then 40 studies, the reported data set is far from complete. I.e., data was curated with the focus on healthy subjects with publications reporting time course information or if no time courses were available high quality pharmacokinetics information. Depending on the question additional studies should be curated to extend the data collection, e.g., pharmacokinetics on drug interactions with simvastatin. By providing the data set in a standardized format via a publicly available database others can reuse the dataset and contribute additional studies to the collection.

A large variability in simvastatin pharmacokinetics exists between individuals and between study cohorts as can be seen by the performed meta-analysis on pharmacokinetics information and the large error-bars on the reported time courses for simvastatin and simvastatin metabolites. These inter-individual and inter-study variability poses a challenge for computational modelling. Despite this variability most studies are in good agreement with other studies as can be seen in the dose-dependency of the pharmacokinetics parameters of simvastatin. The reasons for outliers can in most of the cases be determined and fall in the categories: (i) curation errors, which can and have been fixed; (ii) reporting errors in studies such as incorrect units (which are either obvious and can be fixed or unclear), (iii) special subgroups or study protocols (e.g. certain genotypes, ethnicities, disease or dosing protocols such as slow release tablets). Such data can be filtered from the analysis. Some studies did not report clearly enough what substances were measured and how, making it impossible to curate the corresponding data.

Simvastatin pharmacokinetics model The curated database was used to develop and validate a physiological-based computational model involving absorption, distribution, metabolism and elimination of simvastatin and its metabolites.

This model was parametrized based on research and parameter fitting. The parameter fitting was done using single administration simvastatin timecourses and validated with multiple administration timecourses. The focus of the model was application in healthy subjects and the model was calibrated accordingly with data-sets limited to healthy subjects. Most clinical studies have very homogeneous study cohorts (e.g. age ranges between 20-45 years, Caucasian), so that the resulting model is representative of this study population. The simvastatin model with its default parametrization (reference model) cannot be applied to predict other sub-groups, such as with non-healthy subjects. Reparametrization of the respective parameters is required. Such data sets, with large deviation compared to the healthy studies, had to be removed from the parameter fitting, because with a single parameter-set healthy and non-healthy timecourses could not be predicted simultaneously. Consequently, data for non-healthy subjects an data sets with very large deviation from the other datasets

were excluded from the parameter fitting.

Importantly, all studies could be fit individually, i.e. using data from a study with disease allowed to fit model parameters for the respective disease state. Based on data-sets for certain subgroups, such as diseases or genotypes, specific parameter-sets could be fitted. This would allow predictions about possible changes in parameters for the subgroups compared to healthy controls.

Secondly, simvastatin is affected by many drug-drug interactions which are currently not included in the model. Although the curated data already contains a multitude of information on these drug-drug interactions, no fitting was performed for the respective inducer and inhibitor data. The main focus of this thesis was to create a pharmacokinetic model of simvastatin which can be applied in simvastatin therapy.

The pharmacokinetics model predicted the timecourses of simvastatin, simvastatin acid, active inhibitors and the sum of simvastatin and simvastatin acid in very good agreement with the experimental data. Only timecourses of total HMG-CoA reductase inhibitors showed a systematic offset (all of the simulations predicted to low concentrations). Multiple issues could have contributed to this discrepancy. First of all only very limited data on total HMG-CoA reductase inhibitors were available compared to for instance simvastatin timecourses. The parameter fitting consequently favoured fitting model variables with a large data coverage better. Furthermore, in the model the secondary simvastatin metabolites besides simvastatin acid were pooled as simvastatin metabolites and an inhibitory activity of 50 % was assumed for the simvastatin metabolites. This activity and the assumption of inhibitory activity both affect the model predictions of total HMG-CoA reductase inhibitors. A systematic effect of the inhibitory activity of the simvastatin metabolites on the parameter fitting would be required.

Furthermore many model assumptions were made. Transport reactions did not differ between the transport mechanisms. Simvastatin is able to cross biomembranes via passive diffusion, while its metabolites need to be transported by active membrane-transporters. The passive diffusion of simvastatin was not included and it was assumed that all transportation processes can be described with either an irreversible or reversible Michaelis-Menten kinetic.

It was assumed that esterase mediated conversion of simvastatin to simvastatin acid is only present in the liver. However this conversion can take place in other tissues to some extent as well, especially conversion by plasma esterases.

An important assumption was the pooling of all other simvastatin metabolites that are not simvastatin and simvastatin into a single species. This could have been differentiated more, which may could have lead to better fitting results for total HMG-CoA reductase inhibitors. The complexity of the model was clearly driven by the availability of data in the literature. Without the availability of timecourse information on these secondary metabolites the additional parameters introduced in the model would have been difficult to fit.

In summary, the resulting model of simvastatin pharmacokinetics reproduces and predicts single and multiple application studies with timecourses for simvastatin, simvastatin acid and active HMG-CoA reductase inhibitors in healthy subjects under control conditions. Pharmacokinetic parameters and the dose-dependency were successfully reproduced.

Cholesterol pharmacodynamics model In a next step a simplified cholesterol model was developed and coupled to the pharmacokinetics model of simvastatin. The model includes a model of hepatic homeostasis including cholesterol biosynthesis, fecal loss, dietary uptake and plasma export and uptake. Cholesterol biosynthesis is highly regulated with many reaction steps. Within the model this was simplified to two main steps involving the key enzymes HMG-CoA synthase and reductase. The export of hepatic cholesterol and the uptake from lipoproteins in the blood is abbreviated to a single reaction step, accounting for the export and the uptake in the same way. Biologically cholesterol is initially packed into VLDL, which are converted to IDL and subsequently to LDL. VLDL and IDL as well as HDL contribute to the cholesterol distribution in the body. HDL transports cholesterol back to the liver from peripheral tissues. The main focus of the model was a simplified description of LDL-cholesterol. No triglycerides were included in the model.

Also regulatory mechanisms of LDL-receptors as well as HMG-CoA reductase and synthase are highly simplified (e.g. by using zero order kinetics for synthesis and degradation of the proteins). Overall the focus was on a model able to describe changes in plasma LDL-cholesterol after simvastatin therapy which is in line with reported rates of cholesterol turnover and concentration ranges. Future model extensions would describe the involved processes more realistically and with more detail. A focus should be using separate expression and translation equations for the synthesis and first-order degradation kinetics depending on the actual amount of protein.

The predicted timescale of plasma LDL-cholesterol reduction in simvastatin treatment was too slow. Within this work only minimal adjustments of the cholesterol model parameters were performed. No automatic calibration of the model based on experimental data was applied.

Pharmacokinetics/pharmacodynamics model The complete whole model pharmacokinetics/pharmacodynamcis model including whole-body cholesterol and the simvastatin model is able to predict the mechanisms and effects of simvastatin on plasma LDL-cholesterol levels and the HMG-CoA reductase in line with experimental data.

Especially the slow decrease in LDL-cholesterol over weeks under daily simvastatin treatment was successfully recapitulated by the model. The model predicted the percentage changes under different doses of simvastatin very well. A very interesting feature of the model is the combination of very fast changes in plasma simvastatin with half-lifes in the range of hours, resulting in fast inhibition of HMG-CoA reductase via competitive inhibition, with slow adaptations of protein amounts (up-regulation of LDL-receptors) and changes in plasma LDL-cholesterol in the range of weeks.

A limitation of the LDL-cholesterol prediction were that the decrease of LDL-cholesterol over the time compared to the data was too slow. The model predicted that the maximum decrease was reached after 10-12 weeks, while the data showed 6-8 weeks. Importantly, due to time-restrictions no fitting of cholesterol parameters based on the LDL-cholesterol decrease was performed, but parameters were manually adjusted. The presented cholesterol model is a first proof-of-concept model for the underlying mechanisms following simvastatin therapy, but has not been optimized towards the experimental data.

In addition, the synthesis and degradation rates of the proteins HMG-CoA synthase and reductase and of the LDL-receptor were assumed to be the identical. A more detailed differentiation and the addition of additional data sets for long-term cholesterol studies would have allowed to fit the correct time scale of LDL-cholesterol decrease.

The established model allowed to study the effects of simvastatin on LDL-cholesterol, e.g., to evaluate the role of dosing intervals and administered simvastatin dose. It needs to be considered that only healthy subjects were included in the parametrization of the simvastatin model but the model was still used to study the effects at elevated plasma LDL-cholesterol levels. From our analysis we could not see any changes in simvastatin pharmacokinetics with plasma cholesterol levels, but if pharmacokinetics of simvastatin differ in hypercholesterolemic subjects this would have an effect on the model predictions.

Many hypercholesterolemic subjects take multiple medications in addition to simvastatin (e.g. medication against hypertension or diabetes). Such polypharmacy could via drug-drug interactions based on enzyme induction or inhibition change the pharmacokinetics of simvastatin. All model predictions only consider simvastatin application without any co-medication.

In line with our initial hypothesis (sec. 1.4), we were able to create a pharmacokinetic/pharmacodynamic model of simvastatin and cholesterol including the inhibition of HMG-CoA reductase and the transcriptional/translational regulation of HMG-CoA synthase, HMG-CoA reductase and LDL-receptors. Using the model, we were able to explain the effects of simvastatin on HMG-CoA reductase and cholesterol level.

5 Outlook

Application to special cohorts Important next steps are the application and adaption of the model to special cohorts. Future simvastatin model variants would provide parametrization based on studies in non-healthy and in older patients. This would especially be of importance, because LDL-cholesterol levels are often found in patients of higher age.

A special group of interest are patients with homo- or heterozygote familiar hypercholesterolemia (FH). These patients have mutations affecting the expression or activity of LDL-receptors, which are the mainly responsible for the slow LDL-cholesterol decrease in simvastatin therapy (as also predicted by the presented model). Homozygote FH patients lack LDL-receptors, which would make simvastatin treatment inefficient. LDL-receptors in heterozygote FH patients are less expressed altering the response to simvastatin. The developed model provides an ideal platform to study the effects of changes in LDL-receptor activity and expression on plasma LDL-cholesterol homo- and heterozygote FH.

Drug-drug interactions The presented work focused on simvastatin therapy without co-administration of other drugs. However, studies report that 20% of people who take medications take other drugs as well [42]. The pharmacokinetics of simvastatin and its metabolites is affected by many inducers and inhibitors

CYP3A4 Inhibitors		**CYP3A4 Inducers**
Nelfinavir	Clarithromycin	Gemfibrozil
Verapamil	Erythromycin	Setipiprant
Diltiazem	Cilostazol	Rifampin
Carbamezipine	Grapefruit	St Johns
Itraconazole	Imatinib	Carbamezipine
Amlodipine		Troglitazone

Drug-drug interactions are of special relevance for CYP3A4, which metabolizes a multitude of drugs in the body. One such example for the strong effects of inhibitors is grapefruit juice. It inhibits CYP3A4 enzymes in the small intestine. A study reports effects that increased the C_{max} and AUC_{0-end} value of simvastatin about 9-fold and 16-fold respectively. The same values for simvastatin acid were increased 7-fold [53]. Inducer and inhibitors acting on CYP3A4 or transporters like OATP1B1 can lead to higher plasma concentration of simvastatin and its metabolites than normal. This increases the risk of side effects such as rhabdomyolysis or myopathy.

To study the drug-drug interactions of simvastatin the influence of inducers and inhibitors on the model needs to be evaluated. Based on pharmacokinetics data on the interaction of these substances model parameters could be fitted to the inducer and inhibitor data. This would create parameter-sets describing the effect sized for the various inhibitors and inducers. Such a strategy would allow to predict the effects of polypharmacy and the required adjustments in simvastatin dosing.

6 Supplement

6.1 Simvastatin single dose experiments

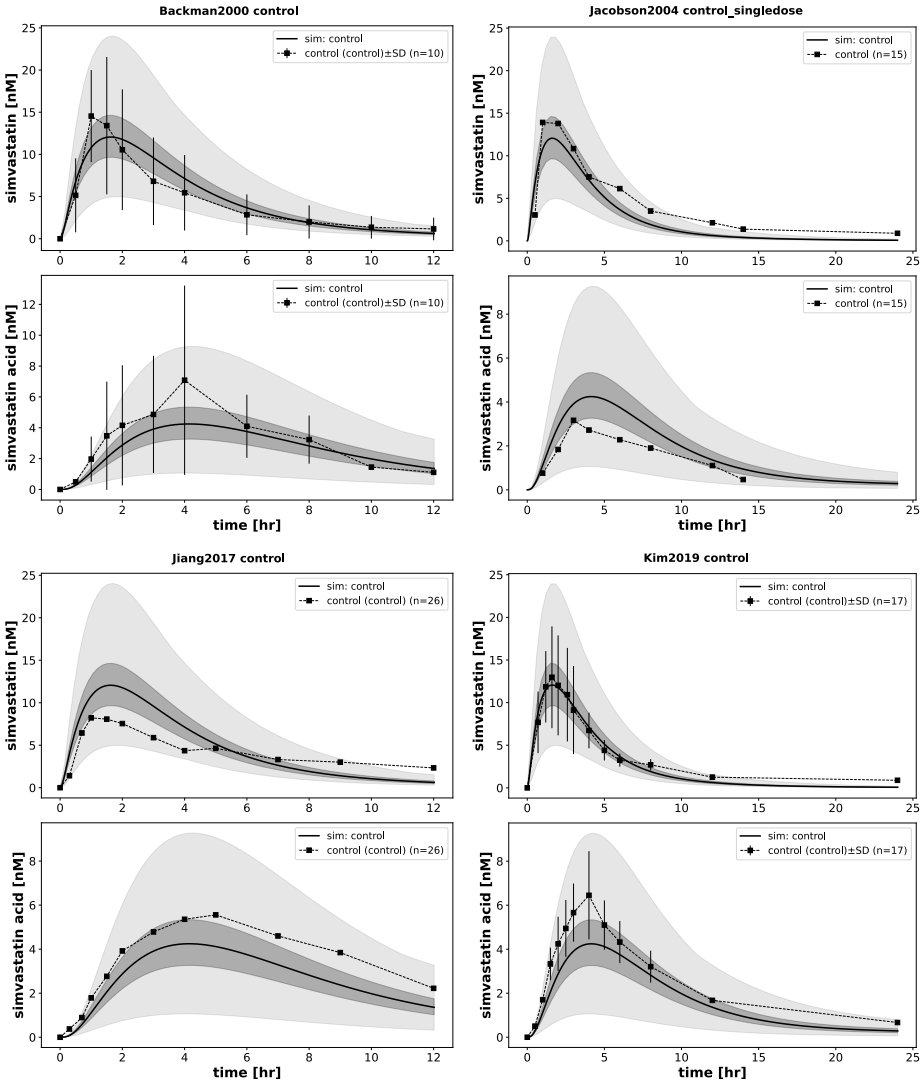


Figure 25: Remainders of the simulation experiments with single dose applications of simvastatin (1/4).

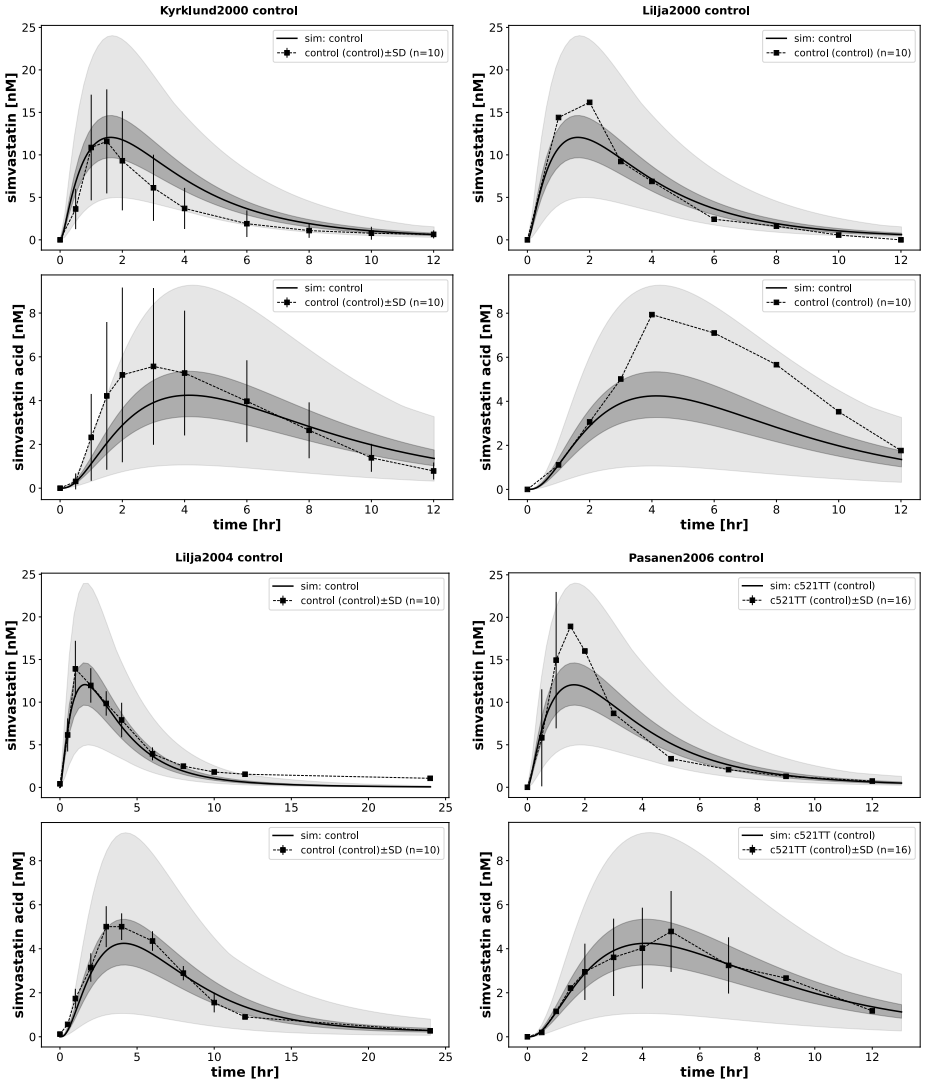


Figure 26: Remainders of the simulation experiments with single dose applications of simvastatin (2/4).

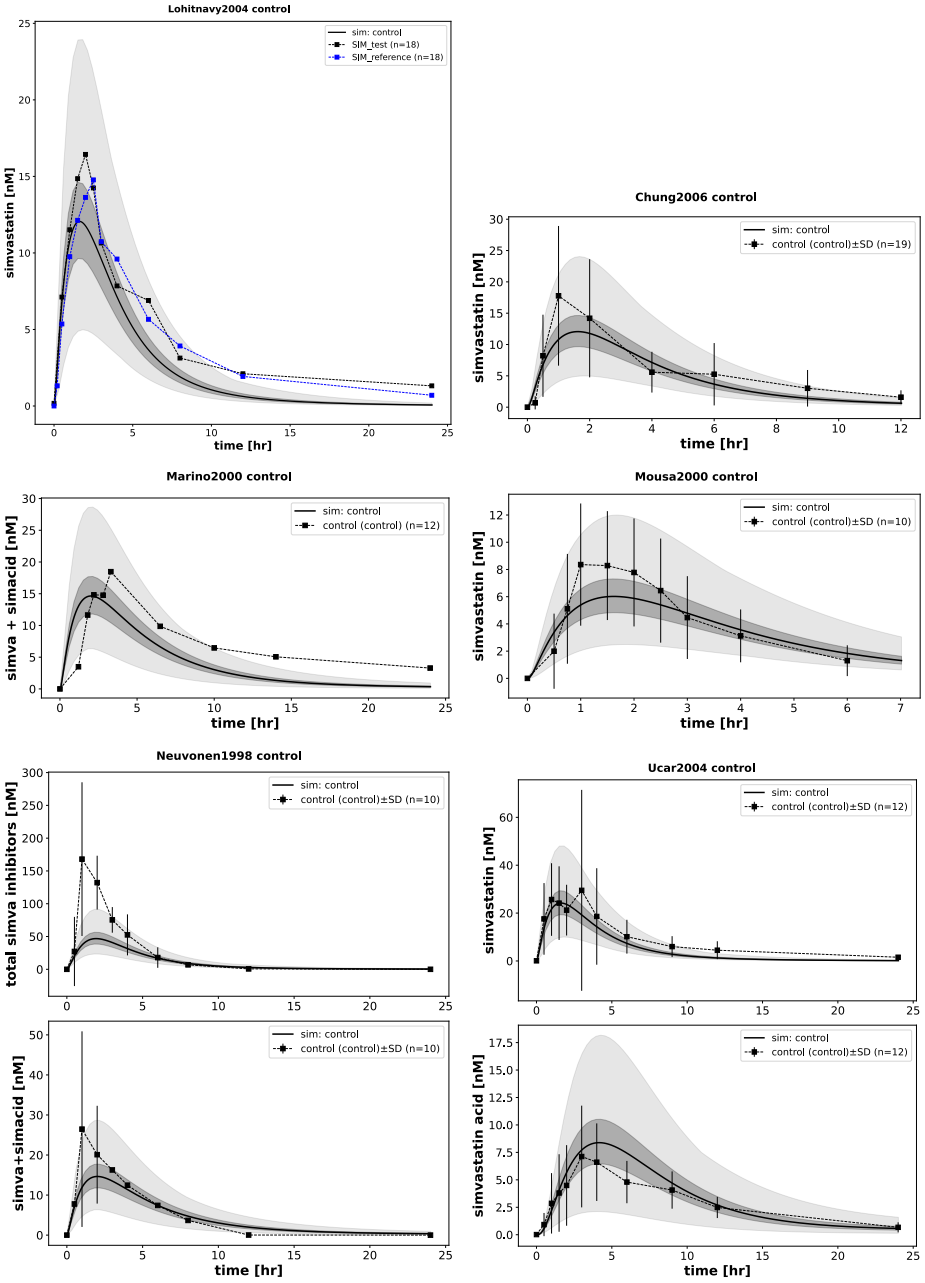


Figure 27: Remainders of the simulation experiments with single dose applications of simvastatin (3/4).

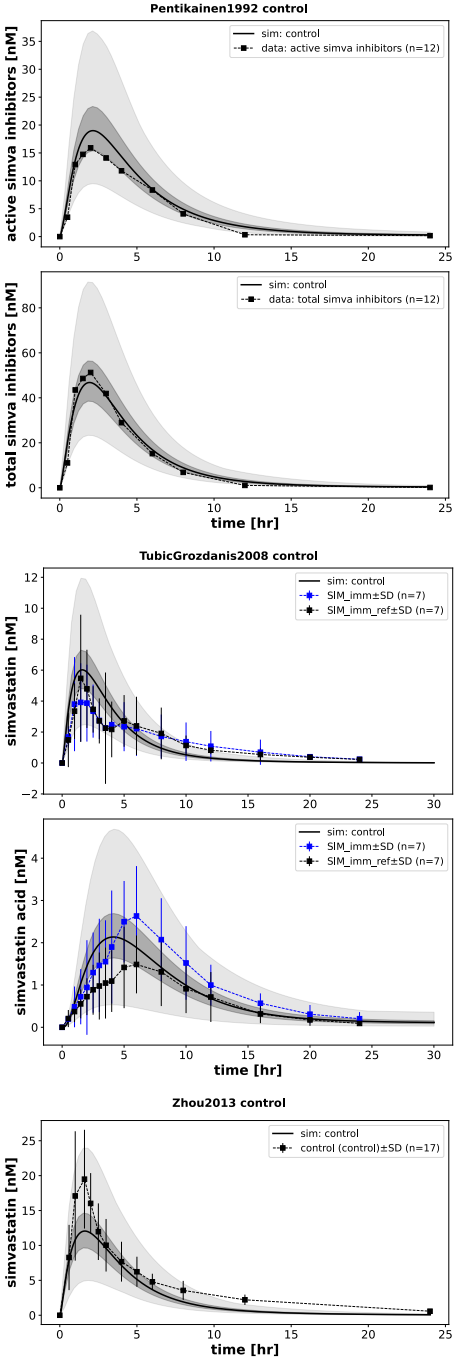


Figure 28: Remainders of the simulation experiments with single dose applications of simvastatin (4/4).

6.2 Simvastatin multiple dose experiments

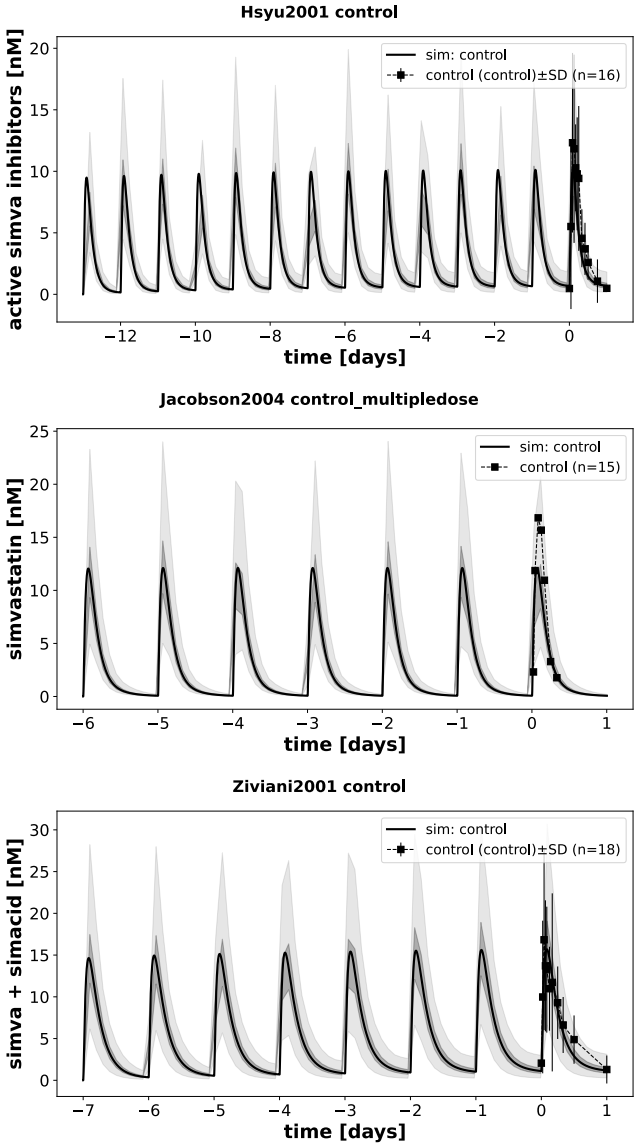


Figure 29: Remainders of the simulation experiments with multiple dose applications of simvastatin.

6.3 Simvastatin pharmacokinetics and meta-analysis

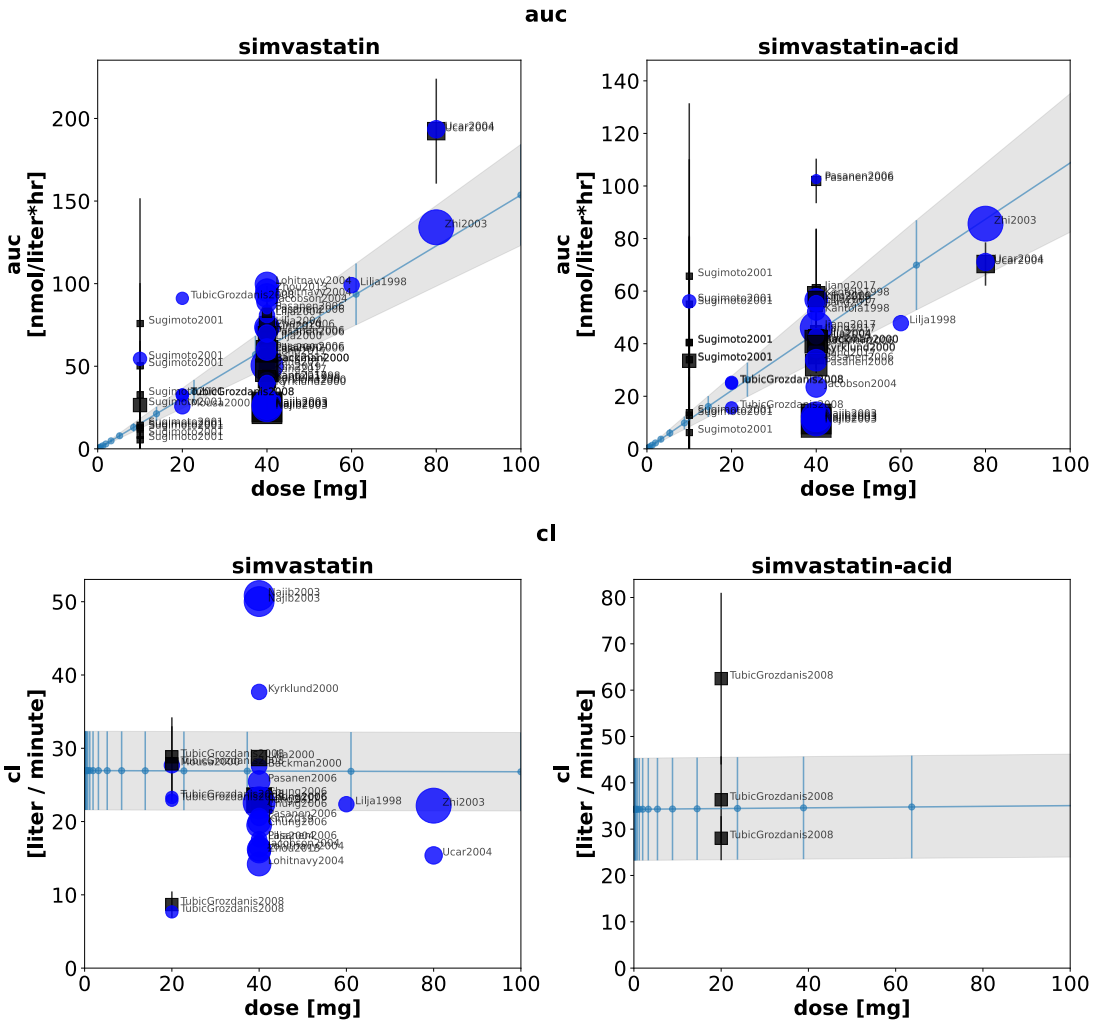


Figure 30: Remainders of the results for the model predictions of the dose-dependency of simvastatin pharmacokinetic parameters AUC and cl. Shown are the dose-dependencies in the range of simvastatin doses from 1 to 100 mg. The sensitivity areas are plotted as the blue shaded areas. Additionally the data from the meta-analysis was added. Datapoints are mean and SD.(1/2)

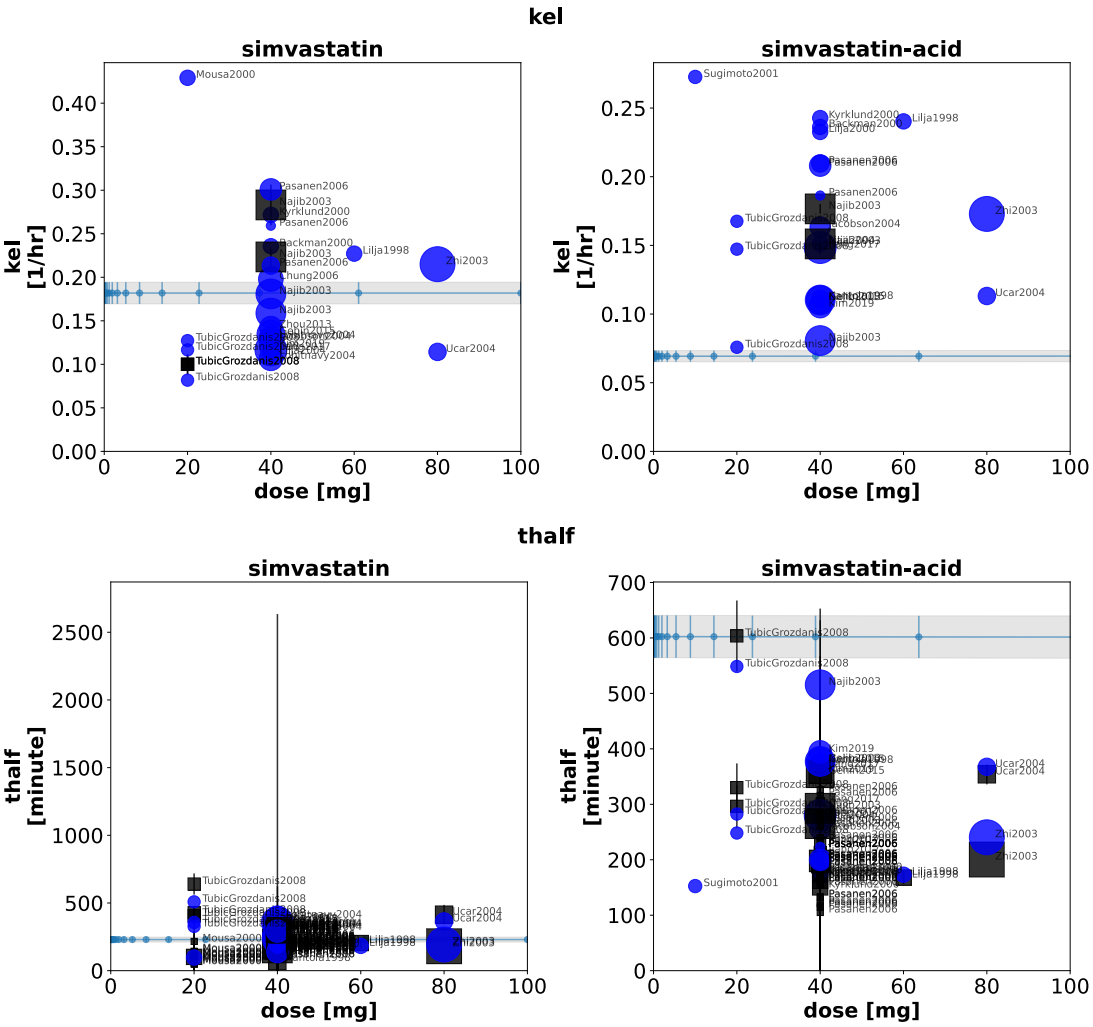


Figure 31: Remainders of the results for the model predictions of the dose-dependency of simvastatin pharmacokinetic parameters kel and $thalf$. Shown are the dose-dependencies in the range of simvastatin doses from 1 to 100 mg. The sensitivity areas are plotted as the blue shaded areas. Additionally the data from the meta-analysis was added. Datapoints are mean and SD. (2/2)

6.4 Cholesterol experiments

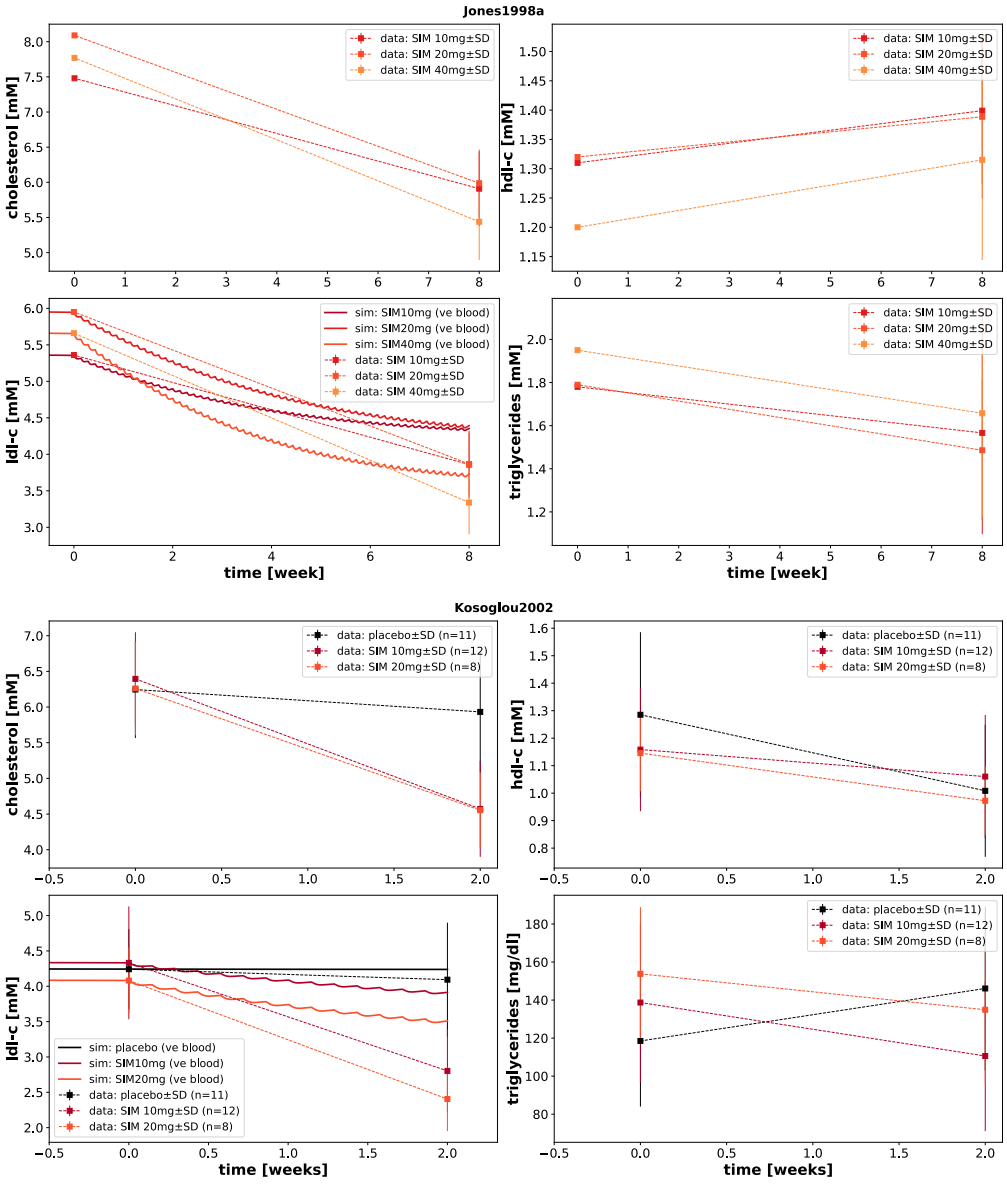


Figure 32: The remaining predicted timecourses of plasma lipids after multiple dose applications of simvastatin (1/2).

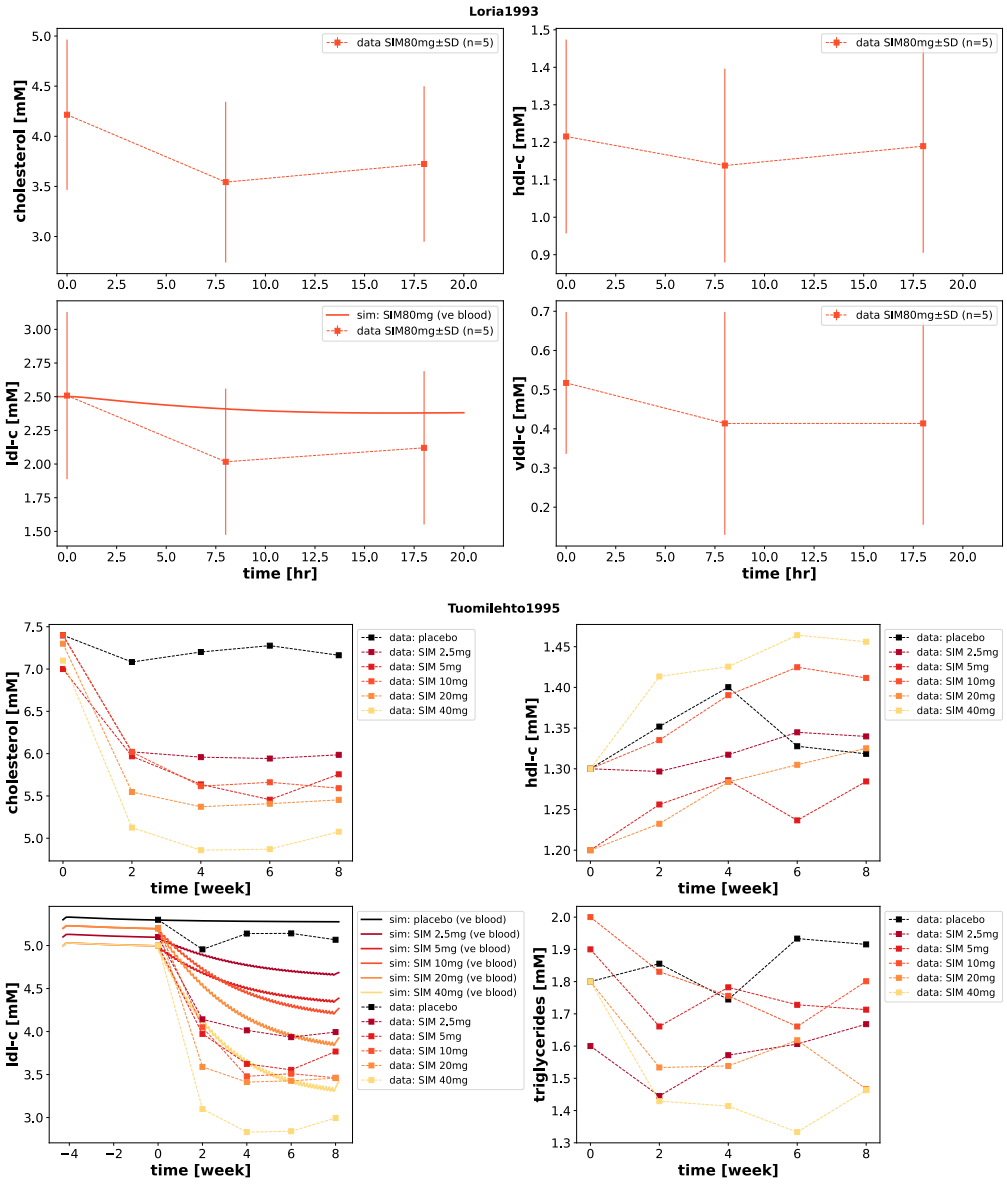


Figure 33: The remaining predicted timecourses of plasma lipids after multiple dose applications of simvastatin (2/2).

6.5 LDL-cholesterol change data

Table 7: Plasma LDL-cholesterol changes after daily administration of different simvastatin doses for different durations obtained from literature.

Study	dose	time [weeks]	baseline	value	sd	se	unit	change	change_sd	count
Jones1998a [40]	10mg	7	5.36	3.859200	0.463104	0.055352	mmol/l	-28.00		70
Jones1998a [40]	20mg	7	5.95	3.867500	0.425425	0.060775	mmol/l	-35.00		49
Jones1998a [40]	40mg	7	5.66	3.339400	0.434122	0.055584	mmol/l	-41.00		61
Jones1998a [40]	10mg	0	5.36	5.36	0.463104	0.055352	mmol/l			70
Jones1998a [40]	20mg	0	5.95	5.95	0.425425	0.060775	mmol/l			49
Jones1998a [40]	40mg	0	5.66	5.66	0.434122	0.055584	mmol/l			61
Kosoglou2002 [47]	10mg	2	167.50	108.40	22.516660	6.50	mg/dl	-35.283582		12
Kosoglou2002 [47]	20mg	2	157.80	93.00	17.54	6.20	mg/dl	-41.064639		8
Kosoglou2002 [47]	10mg	0	167.50	167.50			mg/dl			12
Kosoglou2002 [47]	20mg	0	157.80	157.80			mg/dl			8
Tuomilehto1995 [87]	2.5mg	0	5.10	5.10			mmol/l			28
Tuomilehto1995 [87]	2.5mg	2	5.10	4.142224			mmol/l	-18.779924		28
Tuomilehto1995 [87]	2.5mg	4	5.10	4.014139			mmol/l	-21.291395		28
Tuomilehto1995 [87]	2.5mg	6	5.10	3.934831			mmol/l	-22.846445		28
Tuomilehto1995 [87]	2.5mg	8	5.10	3.993791			mmol/l	-21.690369		28
Tuomilehto1995 [87]	5mg	0	5.00	5.00			mmol/l			28
Tuomilehto1995 [87]	5mg	2	5.00	3.973311			mmol/l	-20.533785		28
Tuomilehto1995 [87]	5mg	4	5.00	3.624420			mmol/l	-27.511597		28
Tuomilehto1995 [87]	5mg	6	5.00	3.554651			mmol/l	-28.906990		28
Tuomilehto1995 [87]	5mg	8	5.00	3.767964			mmol/l	-24.640728		28
Tuomilehto1995 [87]	10mg	0	5.20	5.20			mmol/l			27
Tuomilehto1995 [87]	10mg	2	5.20	4.049283			mmol/l	-22.129173		27
Tuomilehto1995 [87]	10mg	4	5.20	3.479129			mmol/l	-33.093678		27
Tuomilehto1995 [87]	10mg	6	5.20	3.510235			mmol/l	-32.495472		27
Tuomilehto1995 [87]	10mg	8	5.20	3.462529			mmol/l	-33.412907		27
Tuomilehto1995 [87]	20mg	0	5.20	5.20			mmol/l			26
Tuomilehto1995 [87]	20mg	2	5.20	3.588992			mmol/l	-30.980928		26
Tuomilehto1995 [87]	20mg	4	5.20	3.412773			mmol/l	-34.369750		26
Tuomilehto1995 [87]	20mg	6	5.20	3.427311			mmol/l	-34.090180		26
Tuomilehto1995 [87]	20mg	8	5.20	3.458400			mmol/l	-33.492313		26
Tuomilehto1995 [87]	40mg	0	5.00	5.00			mmol/l			29
Tuomilehto1995 [87]	40mg	2	5.00	3.100071			mmol/l	-37.998570		29
Tuomilehto1995 [87]	40mg	4	5.00	2.830967			mmol/l	-43.380653		29
Tuomilehto1995 [87]	40mg	6	5.00	2.840942			mmol/l	-43.181168		29
Tuomilehto1995 [87]	40mg	8	5.00	2.994430			mmol/l	-40.111404		29
Walker1990 [94]	2.5mg	0	226.90	226.90			mg/dl			32
Walker1990 [94]	5mg	0	227.40	227.40			mg/dl			32
Walker1990 [94]	10mg	0	224.80	224.80			mg/dl			32
Walker1990 [94]	20mg	0	230.90	230.90			mg/dl			32
Walker1990 [94]	2.5mg	4	226.90	174.60			mg/dl	-22.50		32
Walker1990 [94]	5mg	4	227.40	167.90			mg/dl	-26.80		32
Walker1990 [94]	10mg	4	224.80	153.70			mg/dl	-30.90		32
Walker1990 [94]	20mg	4	230.90	144.10			mg/dl	-37.40		32
Aranda1994 [73]	10mg	26						-23.00		38
Aranda1994 [73]	20mg	26						-28.00		37
Keech1994 [43]	20mg	8						-38.00		208
Keech1994 [43]	40mg	8						-41.00		206
Marshall1994 [63]	10mg	12						-29.00		41
Marshall1994 [63]	20mg	12						-36.00		41
Marshall1994 [63]	40mg	12						-41.00		41
Mol1986 [66]	2.5mg	4						-5.50	7.4	8
Mol1986 [66]	5mg	4						-17.70	-26.5	4
Mol1986 [66]	10mg	4						-27.70	6.5	8
Mol1986 [66]	20mg	4						-30.40	13.6	4
Mol1986 [66]	40mg	4						-37.00	6.5	7
Mol1986 [66]	80mg	4						-42.20	6.9	4
Mol1988 [67]	20mg	4						-35.50	8.9	38
Mol1988 [67]	20mg	8						-38.30	8.7	38
Mol1988 [67]	40mg	12						-44.40	8.5	38
Mol1988 [67]	40mg	16						-44.20	9.0	38
Mol1988 [67]	40mg	20						-45.30	9.5	38
Mol1988 [67]	40mg	24						-42.50	10.5	38
Ntanios1999 [71]	40	24 7.69	3.916160	1.797720			-49.074642		7	
Ntanios1999 [71]	80	24 8.12	4.152480	1.844140			-48.861084		11	

6.6 Reference concentrations and parameters

Table 8: Table of reference concentrations obtained from literature. The bold lines were used in the model.

study	pmid/doi	type	species	tissue	substrate	mean	sd	min	max	unit
Corkey1988	3417871	concentration	human	liver microsomes	CoASH	154.00	35.0			nmol/g
Corkey1988	**3417871**	**concentration**	**human**	**liver microsomes**	**acetyl-CoA**	**43.00**	**31.0**			**nmol/g**
Corkey1988	3417871	concentration	human	liver microsomes	Methylmalonl CoA			2.0	nmol/g	
Corkey1988	3417871	concentration	human	liver microsomes	Succinyl CoA	42.00	36.0			nmol/g
Corkey1988	**3417871**	**concentration**	**human**	**liver microsomes**	**HMG-CoA**	**22.00**	**27.0**			**nmol/g**
Corkey1988	3417871	concentration	human	liver microsomes	Propionyl CoA				2.0	nmol/g
Corkey1988	3417871	concentration	human	liver microsomes	Isobutyryl CoA				2.0	nmol/g
Corkey1988	3417871	concentration	human	liver microsomes	Isovaleryl CoA				2.0	nmol/g
Corkey1988	3417871	concentration	human	liver microsomes	Octanoyl CoA				2.0	nmol/g
Lumbers1969	**4314688**	**concentration**	**rat**	**liver**	**acetyl-CoA**			**28.00**	**34.5**	**nmol/g**
Lumbers1969	**4314689**	**concentration**	**rat**	**liver**	**acetyl-CoA**			**62.70**	**81.5**	**nmol/g**
Lumbers1969	4314690	concentration	rat	liver	acetyl-CoA			16.10	103.0	nmol/g
Makino2016	27582390	concentration	human	liver	total free cholesterol			40.00	45.0	nmol/g
Chuang2017	28322747	concentration	mouse	liver	total free cholesterol			2.14	59.2	nmol/g
Chuang2017	**28322747**	**concentration**	**mouse**	**liver**	**total free cholesterol**			**5.80**	**160.0**	**nmol/g**
Rudling1997	9151797	concentration	rat	liver	total free cholesterol	3.00				nmol/g
Rudling1997	**9151797**	**concentration**	**rat**	**liver**	**total free cholesterol**	**8.17**				**nmol/g**
Villarasa2019	31108462	concentration	mouse	liver mitochondria	total free cholesterol	4.00				mg/g
Vilarrasa2019	**31108462**	**concentration**	**mouse**	**liver mitochondria**	**total free cholesterol**	**10.00**				**nmol/g**

Table 8: Table of reference parameters obtained from literature. The lines with studies marked bold were used in the model. No PMIDs were found for the studies Clinkenbeard1975 and Espenshade2013, however the corresponding DOIs are: 10.1016/0076-6879(75)35151-3 and 10.1016/B978-0-12-378630-2.00070-0, respectively.

study	pmid	enzyme	parameter	species	tissue	substrate/inhibitor	product	mean	sd	min	max	unit	count
Chen2005	15616150	OATP1B1	IC50					3.600				μM	
Chen2005	15616150	OATP1B1	IC50			simvastatin lactone		9.700				μM	
Ishigam2001	11465417		Km	homo sapiens	liver microsomes		simvastatin metabolites	5.250	1.24			μM	
Ishigam2001	11465417		Km	homo sapiens	liver microsomes		simvastatin metabolites	5.050	0.55			μM	
Ishigam2001	11465417		Km	homo sapiens	liver microsomes		simvastatin metabolites	2.110	0.87			μM	
Ishigam2001	11465417		Km	homo sapiens	liver microsomes		simvastatin metabolites	6.810	1.99			μM	
Prueksaritanont1997	9321523		Km	homo sapiens	liver microsomes	simvastatin	3'-hydroxy simvastatin	20.900	7.80			μM	
Prueksaritanont1997	9321523		Km	homo sapiens	liver microsomes	simvastatin	6'-exomethylene simvastatin	36.200	15.50			μM	
Prueksaritanont1997	9321523		Km	homo sapiens	liver microsomes	simvastatin	3',5'-dihydrodial simvastatin	35.000	5.60			μM	
Prueksaritanont2003	12848784		Km	homo sapiens	liver microsomes	simvastatin acid	simvastatin metabolites	76.000	35.00			μM	
Prueksaritanont2003	12848784		Km	homo sapiens	liver microsomes	simvastatin acid	simvastatin metabolites	47.000	12.00			μM	
Prueksaritanont2003	12848784		Km	homo sapiens	liver microsomes	simvastatin acid	simvastatin metabolites	47.000	21.00			μM	
Prueksaritanont2003	12848784	CYP3A4	Km			simvastatin acid	simvastatin metabolites	26.000				μM	
Prueksaritanont2003	12848784	CYP3A4	Km			simvastatin acid	simvastatin metabolites	29.000				μM	
Prueksaritanont2003	12848784	CYP3A4	Km			simvastatin acid	simvastatin metabolites	21.000				μM	
Prueksaritanont2003	12848784	CYP2C6	Km			simvastatin acid	simvastatin metabolites	88.000				μM	
Prueksaritanont2003	12848784	CYP2C7	Km			simvastatin acid	simvastatin metabolites	36.000				μM	
Prueksaritanont2003	12848784	CYP2C8	Km			simvastatin acid	simvastatin metabolites	16.000				μM	
Li2019	30776567	CES1b	Km	homo sapiens	plasma	simvastatin	simvastatin acid	87.400				μM	3.0
Li2019	30776568	CES1c	Km	homo sapiens	plasma	simvastatin	simvastatin acid	353.400				μM	3.0
Li2019	30776569	PON1	Km	homo sapiens	plasma	simvastatin	simvastatin acid	103.010				μM	3.0
Li2019	30776570	PON3	Km	homo sapiens	plasma	simvastatin	simvastatin acid	337.620				μM	3.0
Fukami2010	20810539	CES2	Ki	homo sapiens	liver microsomes	simvastatin		0.670	0.09			μM	
Fukami2010	20810539	CES1A1	Ki	homo sapiens	liver microsomes	simvastatin		0.800	0.10			μM	
Fukami2010	20810539	CES2	IC50	homo sapiens	liver microsomes	simvastatin		0.780				μM	
Fukami2010	20810539	CES1A1	IC50	homo sapiens	liver microsomes	simvastatin		0.760				μM	
Clinkenbeard1975		HMG-CoA synthase	Km	chicken	liver	acetoacetyl-CoA					2.5	μM	
Clinkenbeard1975		HMG-CoA synthase	Km	chicken	liver	Acetyl-CoA		0.300				mM	
Espenshade2013		HMG-CoA reductase	Ki			Mevinolin		1.000				nM	
Espenshade2013		HMG-CoA reductase	Km			HMG-CoA		1.000				μM	
DaCosta 2011	22159045	HMG-CoA reductase	Ki			simvastatin				4.3	18.0	nM	
Istvan2001	11349148	HMG-CoA reductase	Km			HMG-CoA		4.000				μM	
Hulcher1973	4147523	HMG-CoA reductase	Kapp			HMG-CoA		0.175				μM	
Hulcher1973	4147523	HMG-CoA reductase	Kapp			NADPH		68.100				mM	
Corsini1995	7784310	HMG-CoA reductase	Ki			simvastatin		0.120				nM	
Corsini1995	7784310	HMG-CoA reductase	Ki			mevastatin		1.400				nM	
Corsini1995	7784310	HMG-CoA reductase	Ki			lovastatin		0.600				nM	
Corsini1995	7784310	HMG-CoA reductase	Ki			pravastatin		2.300				nM	
Corsini1995	7784310	HMG-CoA reductase	Ki			fluvastatin		0.300				nM	
Corsini1995	7784310	HMG-CoA reductase	Km			HMG-CoA		3.000				μM	
Corsini1995	7784310	HMG-CoA reductase	Ki			statins				0.1	2.3	nM	
Corsini1995	7784310	HMG-CoA reductase	IC50	mouse	lymphocytes	simvastatin		3.600				nM	
Corsini1995	7784310	HMG-CoA reductase	IC50	rat	hepatocytes	simvastatin				3.0	50.0	nM	
Corsini1995	7784310	HMG-CoA reductase	IC50	rat	spleen cells	simvastatin		5.000				nM	
Corsini1995	7784310	HMG-CoA reductase	IC50	homo sapiens	HEP G2	simvastatin				150.0	345.0	nM	
Corsini1995	7784310	HMG-CoA reductase	IC50	homo sapiens	skin fibroblasts	simvastatin				2.7	10.0	nM	
Corsini1995	7784310	HMG-CoA reductase	IC50	mouse	lymphocytes	lovastatin		2.000				nM	
Corsini1995	7784310	HMG-CoA reductase	IC50	rat	hepatocytes	lovastatin				3.0	146.0	nM	
Corsini1995	7784310	HMG-CoA reductase	IC50	rat	spleen cells	lovastatin				3.5	4.5	nM	
Corsini1995	7784310	HMG-CoA reductase	IC50	homo sapiens	HEP G2	lovastatin				24.0	50.0	nM	
Corsini1995	7784310	HMG-CoA reductase	IC50	homo sapiens	skin fibroblasts	lovastatin				4.0	188.0	nM	
Corsini1995	7784310	HMG-CoA reductase	IC50	mouse	lymphocytes	pravastatin		1354.000				nM	
Corsini1995	7784310	HMG-CoA reductase	IC50	rat	hepatocytes	pravastatin				5.0	500.0	nM	
Corsini1995	7784310	HMG-CoA reductase	IC50	rat	spleen cells	pravastatin		158.000				nM	
Corsini1995	7784310	HMG-CoA reductase	IC50	homo sapiens	HEP G2	pravastatin				700.0	2650.0	nM	
Corsini1995	7784310	HMG-CoA reductase	IC50	homo sapiens	skin fibroblasts	pravastatin				452.0	9000.0	nM	
Corsini1995	7784310	HMG-CoA reductase	IC50	rat	hepatocytes	fluvastatin				1.7	52.0	nM	
Corsini1995	7784310	HMG-CoA reductase	IC50	rat	spleen cells	fluvastatin		62.000				nM	
Corsini1995	7784310	HMG-CoA reductase	IC50	homo sapiens	HEP G2	fluvastatin				30.0	43.0	nM	
Corsini1995	7784310	HMG-CoA reductase	IC50	homo sapiens	skin fibroblasts	fluvastatin		8.300				nM	
Mosley1998	2513368	HMG-CoA reductase	IC50	rat	liver	pravastatin		48.100	10.90			nM	6.0
Mosley1998	2513368	HMG-CoA reductase	IC50	rat	liver	lovastatin acid		33.400	12.60			nM	6.0
Mosley1998	2513368	HMG-CoA reductase	IC50	rat	liver	simvastatin acid		18.600	6.00			nM	6.0
Mosley1998	2513368	HMG-CoA reductase	IC50	rat	lens	pravastatin		468.700	42.10			nM	12.0
Mosley1998	2513368	HMG-CoA reductase	IC50	rat	lens	lovastatin acid		4.500	0.70			nM	7.0
Mosley1998	2513368	HMG-CoA reductase	IC50	rat	lens	simvastatin acid		5.200	1.50			nM	6.0

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HUMBOLDT-UNIVERSITÄT ZU BERLIN

Lebenswissenschaftliche Fakultät
Institut für Biologie
Institut für Psychologie
Albrecht Daniel Thaer-Institut für Agrar- und Gartenbauwissenschaften

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