Supplementary Material

More power to OATP1B1: An evaluation of sample size in pharmacogenetic studies using a rosuvastatin PBPK model for intestinal, hepatic and renal transporter-mediated clearances

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Supplementary Methods – Power calculation

Power calculations within the Simcyp Simulator are performed as a post-simulation calculation assuming a parallel study design to compare different populations. The methodology used for a given sample size was based on that defined by Armitage et al. (2002)[1] with the user defining the number of subject per population (N), significance level, and the parameter of interest.

First, the parameter of interest and the populations were defined. $X_{Pop1}$was defined as the parameter of interest (e.g. AUC) for extensive transporters (population 1) and x_{Pop2} was defined as the AUC parameter for poor transporters (population 2). Note x_{Pop1} and x_{Pop2} are always the same parameter. It is assumed that x_{Pop1} and x_{Pop2} have the following normal distributions:

$$x_{Pop1} \sim N(\mu_1, {\sigma_1}^2) \text{ and } x_{Pop2} \sim N(\mu_2, {\sigma_2}^2)$$

Where μ_1 and ${\sigma_1}^2$ are the mean and variance of population 1, and μ_2 and ${\sigma_2}^2$ are the mean and variance for population 2.

To determine the mean and variance of each population, a simulation is first run for each population using the population size entered by the user on screen. The mean and variance is then calculated for the selected parameters using the simulation result. By central limit theorem, if a sample of size n_1 is selected from population 1 and a sample of size n_2 is selected from population 2 then the sample means, $\bar{x}_{pop1}$ and $\bar{x}_{pop2}$, have the following normal distributions:

$$\bar{x}_{pop1} \sim N\left(\mu_1, \frac{{\sigma_1}^2}{n_1}\right) \text{ and } \quad \bar{x}_{pop2} \sim N\left(\mu_2, \frac{{\sigma_2}^2}{n_2}\right).$$

To calculate the power, the null hypothesis that population 2 is equal to population 1 was tested, (i.e. population 2 has the same mean and variance as population 1). To calculate the power to detect a difference in this test, the critical value is first calculated for population 1 at the significance level specified on screen. This is derived by calculating the following probability (for α significance level) depending on whether $\mu_2 > \mu_1$ or $\mu_2 < \mu_1$ as follows:

1) If $\mu_2 > \mu_1$ then the probability that an observed value of the random variable $\bar{X}_{pop1}$ is less than the critical value (c) for signifcance level α is defined as:

$$P(\bar{X} < c) = 1 - \alpha = P\left(Z < \frac{c-\mu_1}{\frac{\sigma_1}{\sqrt{n_1}}}\right) \text{ where } Z \sim N(0,1)$$

Therefore $c = \left(\Phi^{-1}(1-\alpha) * \frac{\sigma_1}{\sqrt{n_1}}\right) + \mu_1$

where ϕ is the standard normal cumulative distribution function.

The power of the study is then calculated for each sample size specificed for population 2 by calcuating the probability of $\bar{X}_{pop1}$ being greater than the critical value:

$$P\left(\bar{X}_{pop2} > c\right) \text{ where } \bar{X}_{pop2} \sim N\left(\mu_2, \frac{{\sigma_2}^2}{n_2}\right).$$

This is equivalent to calculating: $P\left(Z > \frac{c-\mu_2}{\sigma_2/\sqrt{n_2}}\right) = 1 - P\left(Z < \frac{c-\mu_2}{\sigma_2/\sqrt{n_2}}\right)$ where $Z \sim N(0,1)$

2) If $\mu_2 < \mu_1$ then the probability that an observed value of the random variable $\bar{X}_{pop1}$ is less than the critical value c for signifcance level α is

$$P\left(\bar{X}_{pop1} < c\right) = \alpha = P\left(Z < \frac{c-\mu_1}{\frac{\sigma_1}{\sqrt{n_1}}}\right) \text{ where } Z \sim N(0,1),$$

therefore $c = \left(\Phi^{-1}(\alpha) * \frac{\sigma_1}{\sqrt{n_1}}\right) + \mu_1$.

The power of the study is then calculated for each sample size specificed for population 2 by calcuating the probability of $\bar{X}_{pop2}$ being greater than the critical value:

$$P\left(\bar{X}_{pop2} < c\right) \text{ where } \bar{X}_{pop2} \sim N\left(\mu_2, \frac{{\sigma_2}^2}{n_2}\right).$$

This is equivalent to calculating $P\left(Z < \frac{c-\mu_2}{\sigma_2/\sqrt{n_2}}\right)$ where $Z \sim N(0,1)$.

Supplementary Table S1 – Parameter Values Used for the Rosuvastatin Simulations.

Parameter	Value	Reference/Comments
Molecular Weight (g/mol)	481.54	
f_u – experimental	0.107	[2, 3]
Blood-to-plasma ratio (*B:P*) – experimental	0.625	[2, 3]
Log of the octanol:water partition coefficient ($logP_{o:w}$) – experimental	2.4	[4]
Compound type	Monoprotic Acid	Marvin Sketch 5.4.0.1[5]
pKa	4.27	[3]
Main plasma binding protein	HSA	Rosuvastatin Astrazeneca Full Prescribing Information
Absorption	ADAM	
Model		
Caco-2 permeability ($P_{app,caco-2(7.4:7.4)}$ [10^{-6} cm/s])	3.395	[6]
Reference compound	Propranolol	
Reference $P_{app,caco-2(7.4:7.4)}$ [10^{-6} cm/s]	20	[6]
fa – predicted	0.66	Based on Caco-2 data
fa – observed	0.55	[2]
ka (h^{-1}) – predicted	0.35	Based on Caco-2 data
ka (h^{-1}) – observed	0.46 – 0.78	Range: [7, 8]
Distribution		
Model	Full PBPK	
V_{ss} (L/kg) – predicted	0.117	Rodgers and Rowland method; see text for details
V_{ss} (L/kg) – observed	1.73	[2]
Elimination		
CL_{iv} (L/h)	48.78	[2]
CL_{int} (µL/min/mg protein)	17	Calculated using the Retrograde model
CL_R (L/h)	17	Meta-analysis [2, 9, 10]
Transport (active and passive)		
Intestinal efflux		
$CL_{int,\ T,\ BCRP}$ (µL/min/cm^2)	35	
Intestinal BCRP REF (User)	1	
Hepatic Uptake and Efflux Intrinsic Clearance		
$CL_{int,\ T,\ OATP1B1}$ (µL/min/million hepatocytes)	109	See text for details; [11]
Hepatic OATP1B1 REF (User)	1	
$CL_{int,\ T,\ OATP1B3}$ (µL/min/million hepatocytes)	36	See text for details; [11]
Hepatic OATP1B3 REF (User)	1	
$CL_{int,\ T,\ NTCP}$ (µL/min/million hepatocytes)	78	See text for details; [11, 12]
Hepatic NTCP REF (User)	1	
$CL_{int,\ T,\ BCRP}$ (µL/min/million hepatocytes)	1.23	[13]
Hepatic BCRP REF (User)	1	
CL_{bile} (L/h) - predicted	15	Using above data

CL_{bile} (L/h) – observed	4 - 195	[14, 15]
Passive intrinsic clearance at sinusoidal membrane		
$CL_{int,PD}$ (ml/min/million hepatocytes)	0.0025	[16]
Renal Uptake and Efflux Intrinsic Clearance		
$CL_{int,T,BCRP}$ (µL/min/cm^2)	1100	Based on Sensitivity Analysis; See text for details
Renal BCRP REF (User)	1	
$CL_{int,T,OAT3}$ (µL/min/cm^2)	1100	Based on Sensitivity Analysis; See text for details
Renal OAT3 REF (User)	1	
Passive Intrinsic Clearance at Basal Membrane		
$CL_{int,PD}$ (ml/min/ million proximal tubule cells)	0.0984	See text for details; [17]
Passive Intrinsic Clearance at Apical Membrane		
$CL_{int,PD}$ (ml/min/million proximal tubule cells)	0.0984	See text for details; [17]

Supplementary Table S2 – Details of the single-dose clinical studies used for performance verification of the rosuvastatin PBPK model, corresponding to supplementary figures S1-S11.

Study Number	Rosuvastatin Dose (mg)	Age-range (years)	Subject Number	Proportion of Females	Study Duration (hrs)	References
1	10	22-42	11	0	30	[18]
2	10	31-60	18	0	72	[19]
3	20	31-60	9	0	72	[19]
4	40	21-39	36	0.139	96	[20]
5	40	31-60	9	0	72	[19]
6	40	21-51	10	0	96	[2]
7	80	29-51	14	0	72	[21]
8	80	22-44	11	0	72	[22]
9	80	25-56	14	0	100	[18]
10	80	31-60	18	0	72	[19]
11	80	35-47	20	0.15	30	[8]

Supplementary Table S3 – Mean predicted versus observed AUC_{0-48}, C_{max} and T_{max} following oral administration of rosuvastatin at 10, 20, 40 and 80 mg doses. Comparisons were made with observed data from 11 independent clinical studies in healthy volunteers.

Study Number	**References**	**AUC_{0-48} (ng.h/mL)**			**C_{max} (ng/mL)**			**T_{max} (hr)**		
		Observed	***Predicted***	***Fold***	***Observed***	***Predicted***	***Fold***	***Observed***	***Predicted***	***Fold***
1	Cooper *et al.* 2003. CPT, 73 (4): 322-329 (10 mg)[18]	51.2	39.3	**0.77**	5.8	4.19	**0.72**	5	3.68	**0.74**
2	Martin *et al.* 2003. CT, 25(8): 2215-2224 (10 mg)[19]	31.6	40.7	**1.29**	3.75	4.35	**1.16**	5	3.70	**0.74**
3	Martin *et al.* 2003. CT, 25(8): 2215-2224 (20 mg)[19]	56.8	86.47	**1.52**	6.79	8.86	**1.30**	5	3.72	**0.74**
4	Lee *et al.* 2005. CPT, 78: 330-341 (40 mg)[20]	216	160.5	**0.74**	25	17.28	**0.69**	4.14	3.58	**0.87**
5	Martin *et al.* 2003. CT, 25(8): 2215-2224 (40 mg)[19]	98.2	166.9	**1.70**	10.3	17.72	**1.72**	5	3.72	**0.74**
6	Martin *et al*. 2003. CT, 25(10): 2553-2563 (40 mg)[2]	165	162.8	**0.99**	18.8	17.45	**0.93**	5	3.75	**0.75**
7	Cooper *et al.* 2002. EJCP, 58: 527-531 (80 mg)[21]	325	335	**1.03**	41.4	35.06	**0.85**	5	4	**0.80**
8	Cooper *et al.* 2003. EJCP, 59: 51-56 (80 mg)[22]	253	320.4	**1.27**	33.7	34.1	**1.01**	4	3.68	**0.92**
9	Cooper *et al.* 2003. CPT, 73 (4): 322-329 (80 mg)[18]	443	310.6	**0.70**	53.5	33.2	**0.62**	3	3.64	**1.21**
10	Martin *et al.* 2003. CT, 25(8): 2215-2224 (80 mg)[19]	268	325.9	**1.22**	30.1	34.77	**1.16**	5	3.70	**0.74**
11	Schneck *et al.* 2004. CPT, 75: 455-463 (80 mg)[8]	410	352.57	**0.86**	49.5	38.06	**0.77**	4	3.69	**0.92**

Supplementary Table S4 – Comparison of predicted and observed pharmacokinetic parameters for rosuvastatin depending on OATP phenotype. The observed values are derived from Pasanen *et al.* 2007[7]. AUC and C_{max} values are reported as mean ± standard deviation. T_{max} is reported as median ± standard deviation.

OATP1B1 Phenotype	**C_{max} (ng/ml)**		**T_{max} (h)**		**AUC_{0-48h} (ng/ml.h)**	
	Predicted	Observed	Predicted	Observed	Predicted	Observed
Extensive Transporter	4.0 ± 2.41	4.21 ± 2.41	3.8 ± 1.89	5.0 (1.0-5.0)	39.3 ± 23.0	33.7 ± 17.5
Intermediate Transporter	5.0 ± 2.81	6.38 ± 3.20	3.6 ± 1.95	4.0 (2.0-5.0)	47.0 ± 25.3	53.1 ± 22.3
Poor Transporter	5.6 ± 4.33	7.53 ± 1.20	3.4 ± 2.36	5.0 (3.0-5.0)	51.5 ± 37.6	55.6 ± 5.4

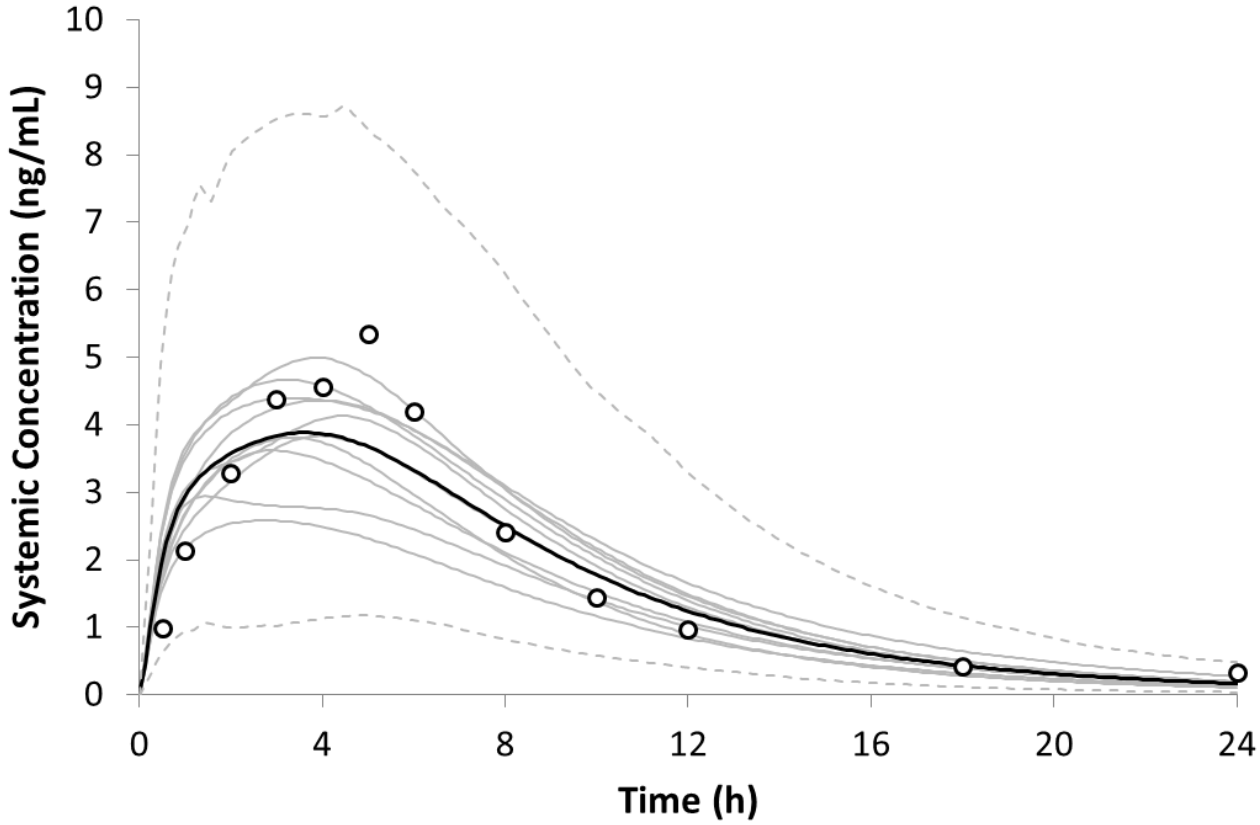

Figure S1 – Simulated plasma concentration-time profiles of rosuvastatin in healthy volunteers following oral administration of 10 mg. The grey thin lines represent simulated individual trials (10) of 11 male subjects (22-42 years) and the solid black line represents the simulated mean of the healthy volunteers population (n=110). The dashed lines represent the upper (5^{th}) and lower (95^{th}) percentiles of simulated concentrations in the total population (n=110 subjects). The circles denote mean values from the clinical study by Cooper *et al.* 2003a[18].

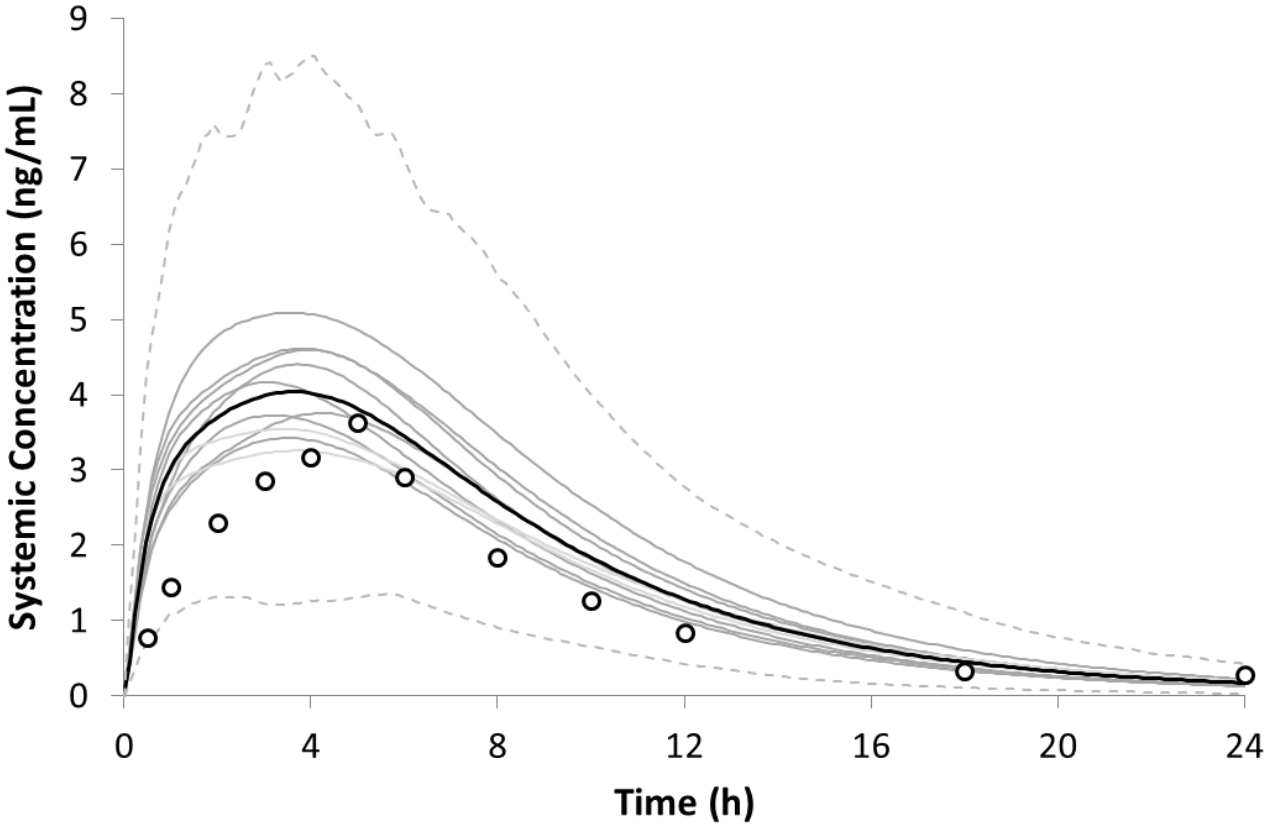

Figure S2 – Simulated plasma concentration-time profiles of rosuvastatin in healthy volunteers following oral administration of 10 mg. The grey thin lines represent simulated individual trials (10) of 18 male subjects (31-60 years) and the solid black line represents the simulated mean of the healthy volunteers population (n=180). The dashed lines represent the upper (5th) and lower (95th) percentiles of simulated concentrations in the total population (n=180 subjects). The circles denote mean values from the clinical study by Martin *et al.* 2003a[19].

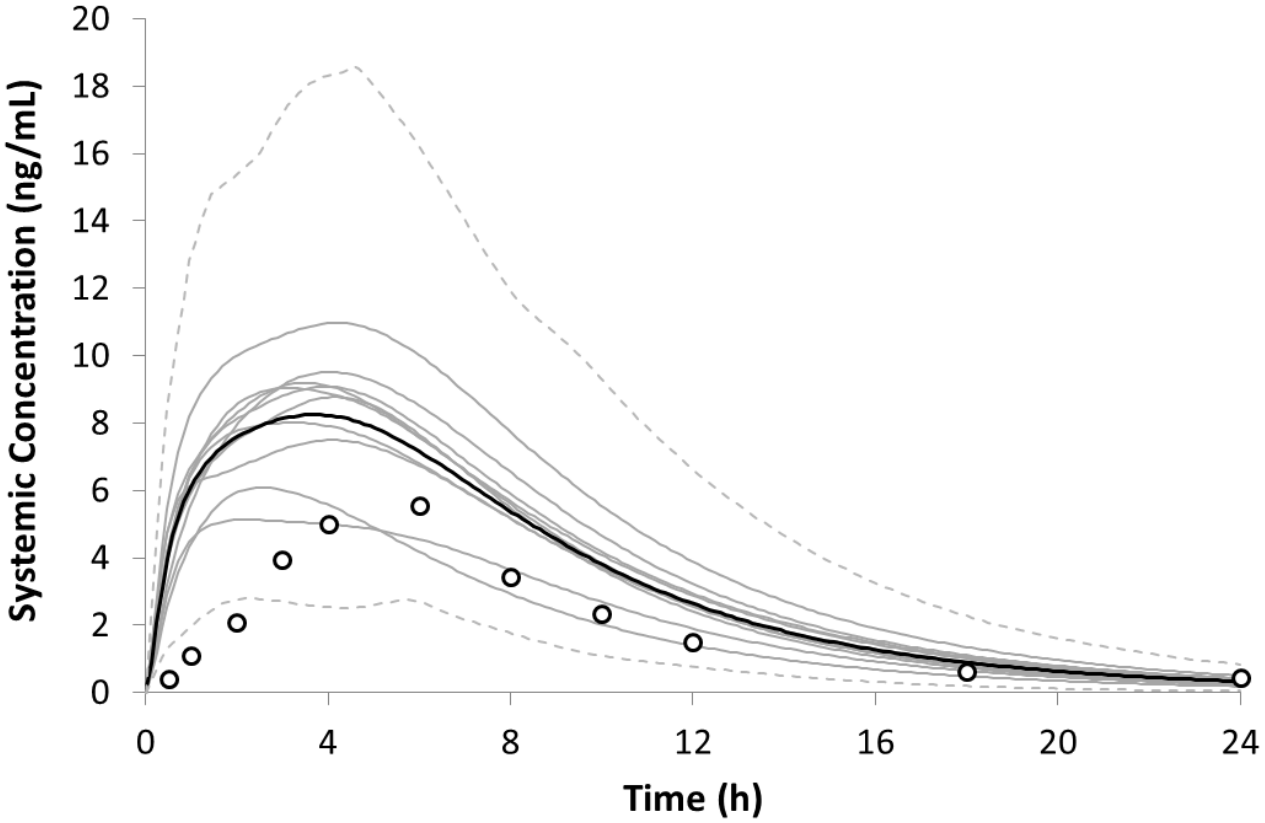

Figure S3 – Simulated plasma concentration-time profiles of rosuvastatin in healthy volunteers following oral administration of 20 mg. The grey thin lines represent simulated individual trials (10) of 9 male subjects (31-60 years) and the solid black line represents the simulated mean of the healthy volunteers population (n=90). The dashed lines represent the upper (5^{th}) and lower (95^{th}) percentiles of simulated concentrations for the total population (n=90 subjects). The circles denote mean values from the clinical study by Martin *et al.* 2003a[19].

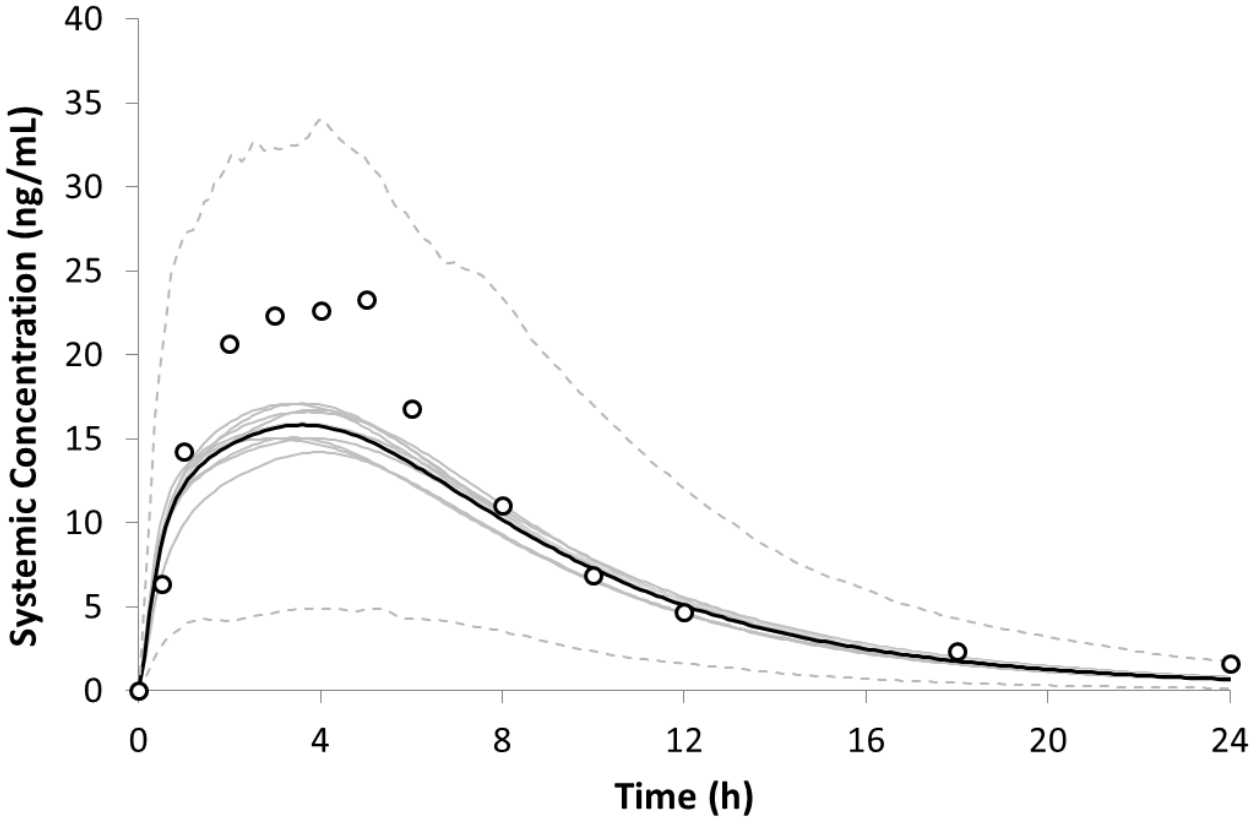

Figure S4 – Simulated plasma concentration-time profiles of rosuvastatin in healthy volunteers following the oral administration of 40 mg. The grey thin lines represent simulated individual trials (10) of 36 subjects (13.9% female, 21-39 years) and the solid black line represents the simulated mean of the healthy volunteers population (n=360). The dashed lines represent the upper (5^{th}) and lower (95^{th}) percentiles of simulated concentrations for the total population (n=360 subjects). The circles denote mean values from the clinical study by Lee *et al.* 2005[20].

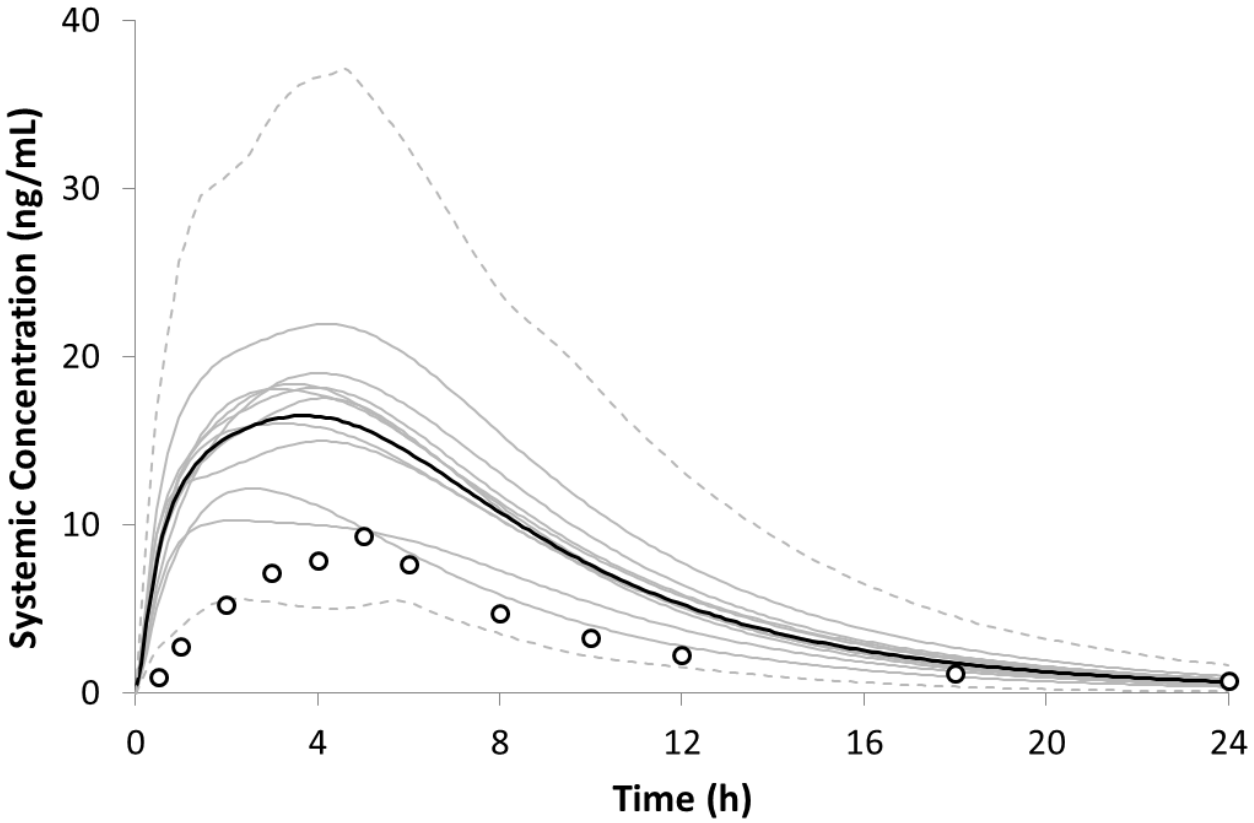

Figure S5 – Simulated plasma concentration-time profiles of rosuvastatin in healthy volunteers following the oral administration of 40 mg. The grey thin lines represent simulated individual trials (10) of 9 male subjects (31-60 years) and the solid black line represents the simulated mean of the healthy volunteers population (n=90). The dashed lines represent the upper (5^{th}) and lower (95^{th}) percentiles of simulated concentrations for the total population (n=90 subjects). The circles denote mean values from the clinical study by Martin *et al.* 2003a[19] .

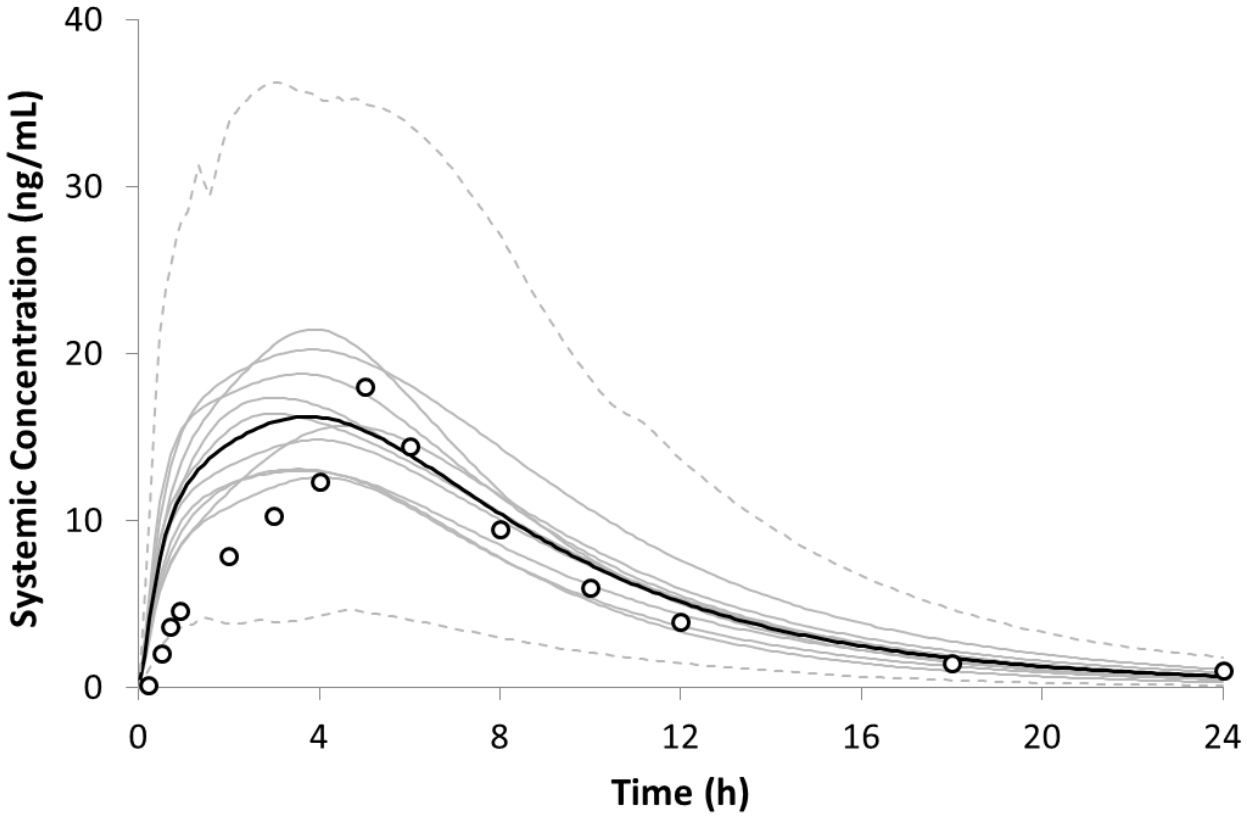

Figure S6 – Simulated plasma concentration-time profiles of rosuvastatin in healthy volunteers following the oral administration of 40 mg. The grey thin lines represent simulated individual trials (10) of 10 male subjects (21-51 years) and the solid black line represents the simulated mean of the healthy volunteers population (n=100). The dashed lines represent the upper (5^{th}) and lower (95^{th}) percentiles of simulated concentrations for the total population (n=100 subjects). The circles denote mean values from the clinical study by Martin *et al.* 2003b[2].

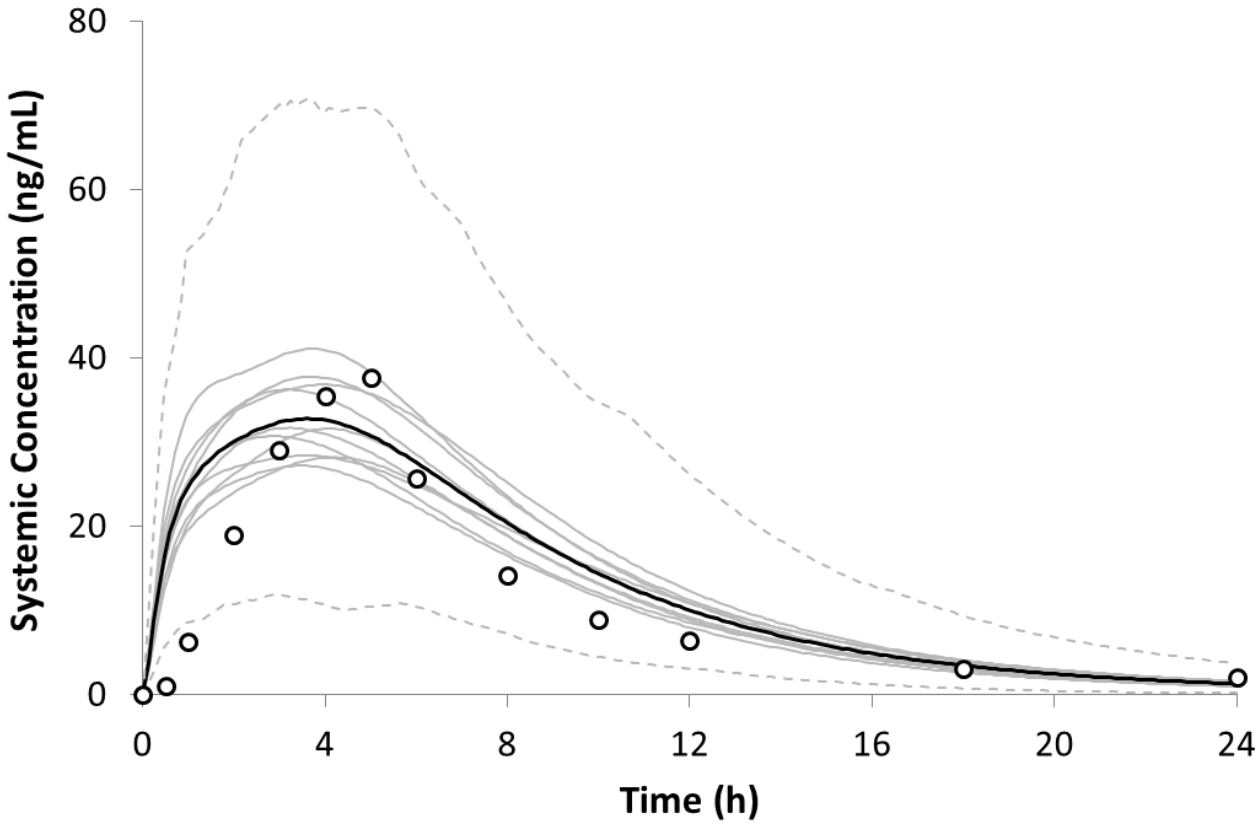

Figure S7 – Simulated plasma concentration-time profiles of rosuvastatin in healthy volunteers following the oral administration of 80 mg. The grey thin lines represent simulated individual trials (10) of 14 male subjects (29-51 years) and the solid black line represents the simulated mean of the healthy volunteers population (n=140). The dashed lines represent the upper (5th) and lower (95th) percentiles of simulated concentrations for the total population (n=140 subjects). The circles denote mean values from the clinical study by Cooper *et al.* 2002[21].

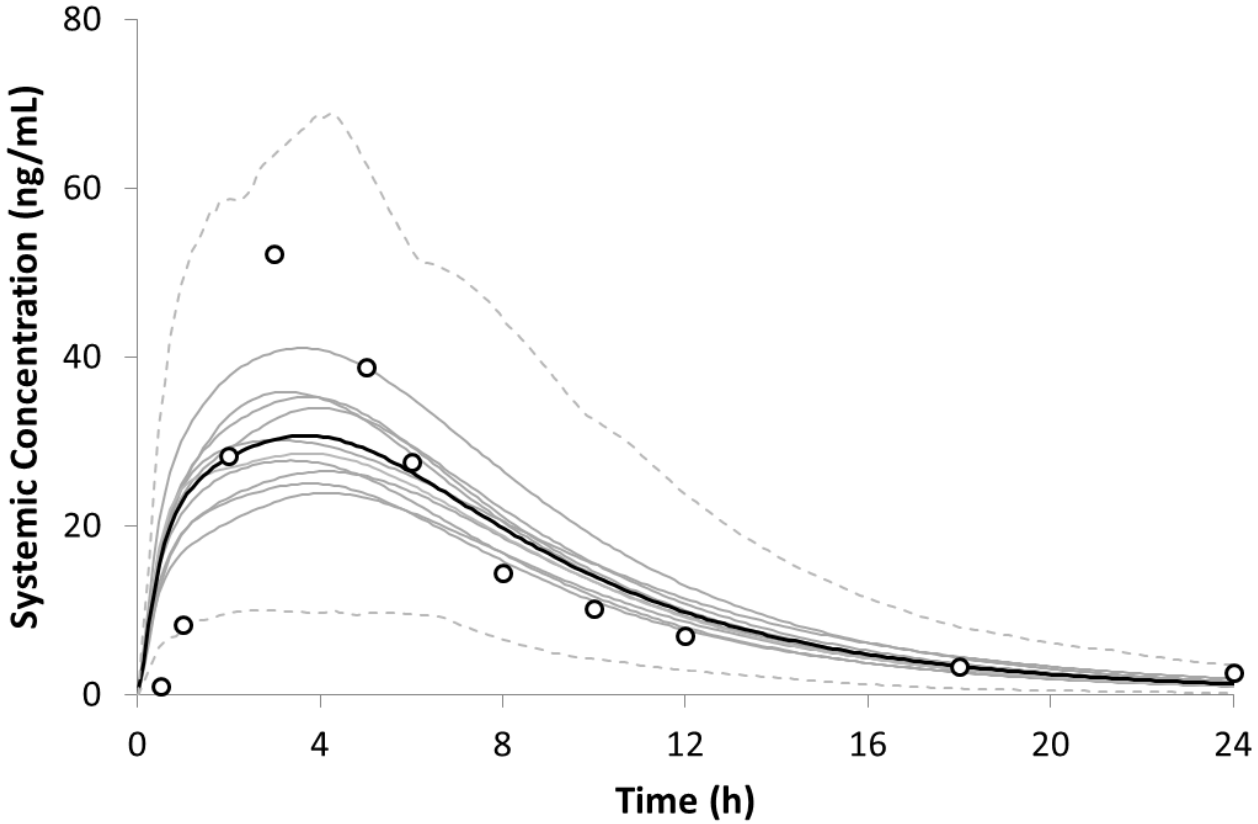

Figure S8 – Simulated plasma concentration-time profiles of rosuvastatin in healthy volunteers following the oral administration of 80 mg. The grey thin lines represent simulated individual trials (10) of 14 male subjects (25-56 years) and the solid black line represents the simulated mean of the healthy volunteers population (n=140). The dashed lines represent the upper (5^{th}) and lower (95^{th}) percentiles of simulated concentrations for the total population (n=140 subjects). The circles denote mean values from the clinical study by Cooper *et al.* 2003b[22].

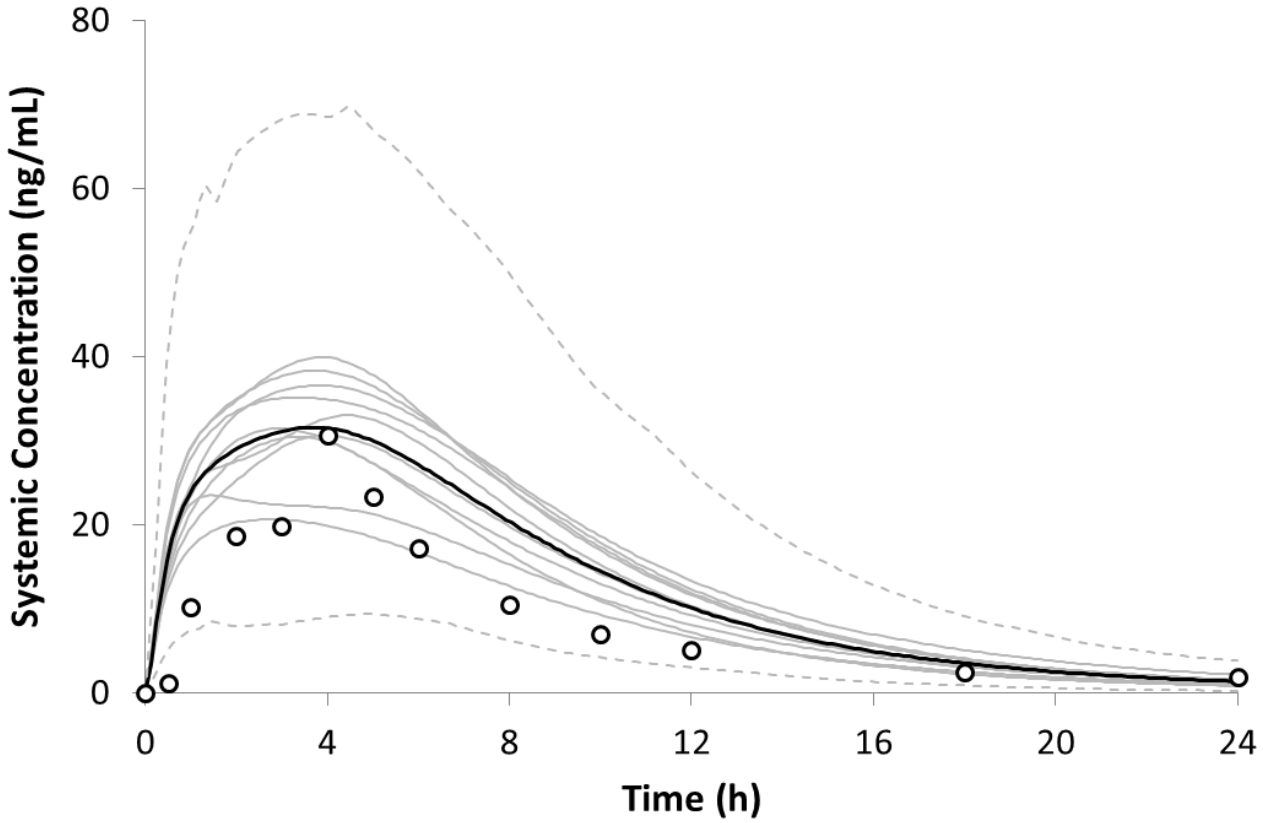

Figure S9 – Simulated plasma concentration-time profiles of rosuvastatin in healthy volunteers following the oral administration of 80 mg. The grey thin lines represent simulated individual trials (10) of 11 male subjects (22-44 years) and the solid black line represents the simulated mean of the healthy volunteers population (n=110). The dashed lines represent the upper (5th) and lower (95th) percentiles of simulated concentrations for the total population (n=110 subjects). The circles denote mean values from the clinical study by Cooper *et al.* 2003a[18].

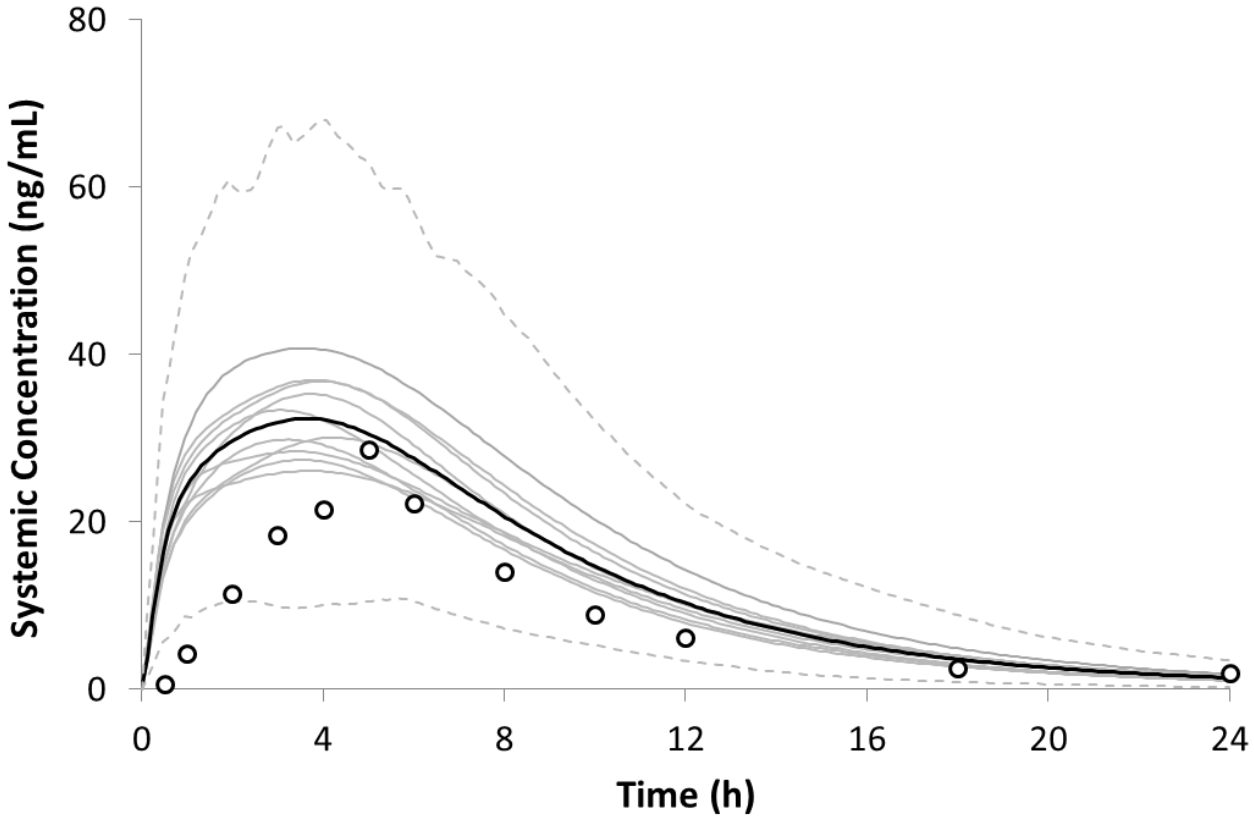

Figure S10 – Simulated plasma concentration-time profiles of rosuvastatin in healthy volunteers following the oral administration of 80 mg. The grey thin lines represent simulated individual trials (10) of 18 male subjects (31-60 years) and the solid black line represents the simulated mean of the healthy volunteers population (n=180). The dashed lines represent the upper (5th) and lower (95th) percentiles of simulated concentrations for the total population (n=180 subjects). The circles denote mean values from the clinical study by Martin *et al.* 2003a[19].

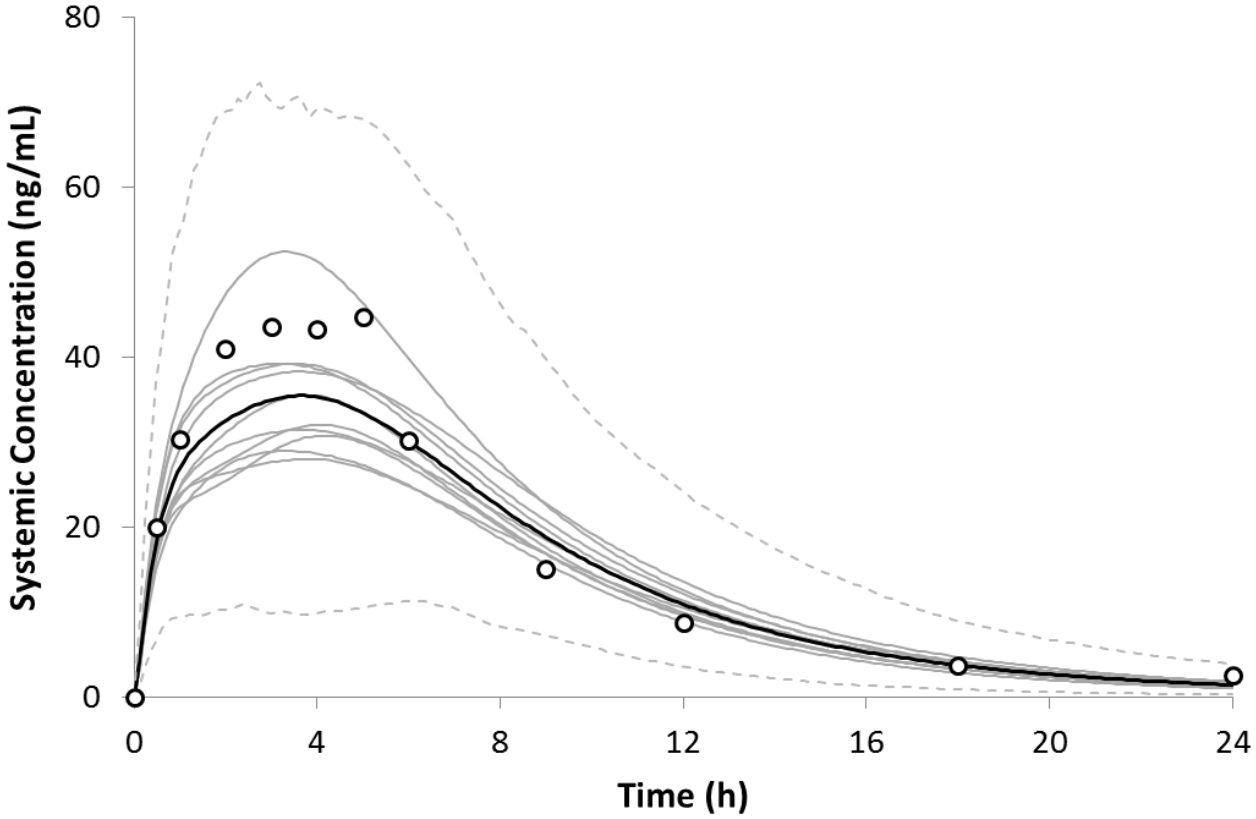

Figure S11 – Simulated plasma concentration-time profiles of rosuvastatin in healthy volunteers following the oral administration of 80 mg. The grey thin lines represent simulated individual trials (10) of 20 subjects (0.15% female, 35-47 years) and the solid black line represents the simulated mean of the healthy volunteers population (n=200). The dashed lines represent the upper (5^{th}) and lower (95^{th}) percentiles of simulated concentrations for the total population (n=200 subjects). The circles denote mean values from the clinical study by Schneck *et al.* 2003[8].

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