A physiologically based in silico kinetic model predicting plasma cholesterol concentrations in humans

Niek C. A. van de Pas, Ruud A. Woutersen, Ben van Ommen, Ivonne M. C. M. Rietjens, and Albert A. de Graaf

The Netherlands Organization for Applied Scientific Research (TNO), 3700 AJ Zeist, The Netherlands; Division of Toxicology, Wageningen University, 6703 HE Wageningen, The Netherlands; and TNO/WUR Centre for Innovative Toxicology, 6703 HE Wageningen, The Netherlands

Abstract Increased plasma cholesterol concentration is associated with increased risk of cardiovascular disease. This study describes the development, validation, and analysis of a physiologically based kinetic (PBK) model for the prediction of plasma cholesterol concentrations in humans. This model was directly adapted from a PBK model for mice by incorporation of the reaction catalyzed by cholesterol ester transfer protein and contained 21 biochemical reactions and eight different cholesterol pools. The model was calibrated using published data for humans and validated by comparing model predictions on plasma cholesterol levels of subjects with 10 different genetic mutations (including familial hypercholesterolemia and Smith-Lemli-Opitz syndrome) with experimental data. Average model predictions on total cholesterol were accurate within 36% of the experimental data, which was within the experimental margin. Sensitivity analysis of the model indicated that the HDL cholesterol (HDL-C) concentration was mainly dependent on hepatic transport of cholesterol to HDL, cholesterol ester transfer from HDL to non-HDL, and hepatic uptake of cholesterol from non-HDL-C. Thus, the presented PBK model is a valid tool to predict the effect of genetic mutations on cholesterol concentrations, opening the way for future studies on the effect of different drugs on cholesterol levels in various subpopulations in silico. A physiologically based in silico kinetic model predicting plasma cholesterol concentrations in humans. J. Lipid Res. 2012. 53: 2734–2746.

Supplementary key words plasma cholesterol • PBK modeling • mutations • LDL cholesterol • HDL cholesterol

In silico modeling has proven to be a useful tool in biology because it allows the study of interspecies variation and regulation of homeostasis and allows the integration of information from various sources (1–3). There are several modeling efforts on cholesterol (4–7), an important biomarker for the risk for cardiovascular events (8–10). Most of these cholesterol modeling studies present models that focus on LDL cholesterol (LDL-C) metabolism in plasma (4–6) or on cellular cholesterol metabolism (7). These models do not represent all relevant components of whole body cholesterol homeostasis because they lack reactions such as cholesterol absorption and biosynthesis in organs, which are the reactions targeted by important cholesterol-lowering drugs, such as statins. This implies that the models cannot fully explain how relevant plasma cholesterol-associated biomarkers are influenced by these drug interventions.

We have, therefore, developed an in silico physiologically based kinetic (PBK) model for plasma cholesterol in the mouse that includes all relevant reactions and that correctly predicts the plasma cholesterol levels of a large variety of mouse strains with gene knockouts related to cholesterol metabolism (11). The model, of which the structure is given in Fig. 1, is able to predict HDL cholesterol (HDL-C), non-HDL cholesterol (non-HDL-C), and total plasma cholesterol (TC) concentrations (12) as well as the intra-organ pools representing hepatic free cholesterol (Liv-FC), peripheral cholesterol (Per-C), intestinal cholesterol ester (Int-CE), hepatic cholesterol ester (Liv-CE), and intestinal free cholesterol (Int-FC) in the mouse. A similar model for humans will be of considerable value in predicting effects of drugs and genetic variations on plasma cholesterol concentrations. Therefore, the aim of this work is to adapt our model to a human version.

Turnover of cholesterol in humans is quantitatively and qualitatively different from that in mice (13). Qualitative difference resides mainly in the protein cholesterol ester

Abbreviations: C, cholesterol (sum of FC and CE); CE, cholesterol ester; CETP, cholesterol ester transfer protein; FC, free cholesterol; FH, familial hypercholesterolemia; GWAS, genome-wide association study; PBK, physiologically based kinetic; SC, sensitivity coefficient; SLOS, Smith-Lemli-Opitz syndrome; TC, total plasma cholesterol (sum of HDL-C and non-HDL-C).

1To whom correspondence should be addressed.

2E-mail: albert.degraaf@tno.nl

3The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of supplementary data, tables and references.
The development of the in silico model was subdivided into the following steps: development of a conceptual model (model structure), mathematical formulation of the model, model calibration, and model validation. After development, a sensitivity analysis was performed to obtain more insight into the factors that determine plasma cholesterol concentrations.

**Conceptual model development**

The conceptual model for the human PBK model was modified from the conceptual model for the mouse (11). Briefly, the mouse model was constructed as follows: relevant knockout mouse models were screened for altered plasma cholesterol levels compared with the levels in the wild-type mouse. If the alteration was more than 2-fold (up or down) compared with the wild-type, the corresponding gene was marked as a key gene. Based on the function of a subset of 12 of these key genes that code for metabolic enzymes producing or consuming cholesterol and transport proteins transporting cholesterol, metabolic and transport reactions were included in the mouse conceptual model (11). The conceptual model for the human was developed from the mouse model by adding human-specific features (see Results). In view of the sparsity of data available to calibrate and validate the model, the number of pools and fluxes in the model was kept to a minimum to avoid overparametrization. As a consequence, FC and CE pools were not distinguished in plasma non-HDL and in the periphery. Also, in the intestine, luminal and enterocytic pools were not distinguished (i.e., the aggregate pools were used).

**Mathematical model formulation**

As the second step in the model development, the conceptual model was converted to mathematical equations. Similar to the mouse model, the human model was formulated as a set of differential equations, each describing the time behavior of one of the cholesterol pools in the conceptual model as a function of the reaction rates.

Equations were slightly altered compared with the mouse model: for the human model, reaction rates were expressed as mmol/man/day, where “man” refers to a standard human, leading to more simple equations (12). For practical reasons, reaction rates (expressed by $v_j$) were numbered according to the numbering in Fig. 1. All symbols of variables and parameters that were used to define the model are given in Table 1. The differential equations, one for each cholesterol pool in the model, are given in Table 2 (Eq. 1–8). Eq. 1, for example, can be interpreted as follows: the change in time of the concentration of hepatic free cholesterol is determined by the balance of the rates of the reactions producing $\left(v_{11}, v_{12}, v_{13}\right)$ and consuming $\left(v_{14}, v_{15}, v_{16}, v_{17}\right)$ hepatic free cholesterol.

In a kinetic model, the reaction rates are calculated using kinetic equations that express the reaction rate as a function of concentrations and kinetic parameters. Most reactions in our model represent a set of lumped enzymatic and/or transport reactions. Often, such a composite reaction can be conveniently represented by a kinetic equation that contains apparent rate constants and apparent $K_M$ values. A general solution is to use Michaelis-Menten kinetics as a prototype kinetic expression for biological reactions. In the present approach, to keep the number of parameters as limited as possible, it was assumed that the reactions operate in the linear part of a Michaelis-Menten kinetic curve (substrate concentration much lower than the apparent $K_M$) or in the saturated part (substrate concentration much higher than the apparent $K_M$). At low substrate concentrations, Michaelis-Menten kinetics reduce to zero-order kinetics (i.e., the reaction becomes independent of the substrate concentration) (Eq. 10; Table 2).
TABLE 1. List of symbols and parameters used

| Symbol | Description | Unit |
|--------|-------------|------|
| $V_i$  | volume of compartment i with i = Liv, Pla, Per, and Int for liver, plasma, periphery, and intestine | L/man |
| $v_i$  | reaction rate of reaction i | mmol/(man·day) |
| $v_{ss}$ | steady state reaction rate of reaction i | mmol/(man·day) |
| $[C]_i$ | tissue or plasma concentration of pool i, with i = Liv-FC, HDL-FC, HDL-CE, non-HDL-C, Per-C, Int-FC, Liv-CE, and Int-CE for liver free cholesterol, HDL free cholesterol, HDL cholesterol ester, non-HDL cholesterol, peripheral cholesterol, intestinal free cholesterol, liver cholesterol ester, and intestinal cholesterol ester | mmol/L |
| $[C]_{ss}$ | steady-state concentration of pool i | mmol/L |
| $k_z$  | zero-order rate constant of reaction i | mmol/(liter·day) |
| $k_f$  | first-order rate constant of reaction i | liter/day |
| $k_s$  | second-order rate constant of reaction i | liter/(mmol·day) |
| $k_{mut,i}$  | $j^\text{th}$-order rate constant of reaction i in a human carrying a mutation that affects reaction i | |
| $k^{*}_i$  | $k^\text{th}$-order reaction rate constant of reaction i that has been altered compared with the normal values | mmol/L |
| $[C]_{ss}^r$  | steady-state concentration of pool i, in case the reaction rate constant of reaction i that has been altered compared with the normal values | |
| $t$  | time | day |
| $f_{mut}$  | rate constant modification factor | [-] |

Reaction 21, catalyzed by CETP, was assumed to have kinetics different from the other reactions. CETP transfers CE from HDL to LDL and requires the binding of both a donor (HDL) and acceptor particle (non-HDL) to exchange cholesterol (20,21). Therefore, the rate of this reaction was dependent on HDL-CE and non-HDL-CE. It was assumed that the reaction rate was linear with respect to both concentrations as given by Eq. 11 (Table 2).

The kinetic formats of reactions 1–20 were obtained from the previously defined mouse model. Instead of developing one optimal model, the mouse model consists of a set (ensemble) of eight submodels, each having a different combination of first- and zero-order kinetics for the various reactions. The model prediction is calculated as the average of the predictions of the submodels. These eight submodels have been selected from a larger set of 65,536 alternative submodels. Each of the suitable submodels has been selected on the basis of a correct prediction of a higher or lower plasma cholesterol level of five knockout mouse strains compared with the wild-type controls (12). For the human situation, not enough data were available to apply an identical selection procedure. Therefore, it was decided to use the kinetic formats of the reactions 1–20 in the eight submodels for the mouse also for the corresponding reactions in the human model. Thus, the eight mouse submodels were converted to eight human submodels, retaining the kinetic orders of reactions 1–20 and adding reaction 21. As in the mouse model, the human model prediction was defined as the average of the predictions of the resulting eight submodels. The predicted TC concentration was calculated as the sum of all three types of plasma cholesterol (non-HDL-C, HDL free cholesterol [HDL-FC], and HDL-CE), whereas the predicted HDL-C concentration was calculated as the sum of HDL-FC and HDL-CE.

Model calibration

The model was calibrated by assigning values to the kinetic parameters ($k_z$, $k_f$, and $k_s$). The values of the parameters were calculated from steady-state concentrations of cholesterol pools and reaction rates of the reactions obtained from literature. Literature data on reaction rates and pool sizes were taken from a wide range of experiments including cannulations, dietary surveys, and in vitro tests, as explained in detail below. Regarding reaction rates, we concentrated on data from experiments that had directly assessed rates that were attributable to a specific reaction in our model.

As usual in PBK modeling (3, 19), the model was developed for a standard human, for which we chose the 70 kg “reference man” as defined by the International Commission on Radiation Protection (19). Thus, data on 70 kg adult male subjects were taken as much as possible. In case the data were obtained from subjects with different body masses, reaction rates were normalized per unit of organ volume and multiplied by the organ mass of the reference man to obtain the reaction rate for the standard human.

If a specific submodel a rate was calculated for a first-order reaction, the corresponding rate constant was calculated using the pool sizes and steady-state reaction rates according to Eq. 12 (Table 2). In the case of a zero-order reaction, the corresponding rate constant was calculated according to Eq. 13 (Table 2). To calculate the rate constant of reaction 21, Eq. 14 was used (Table 2).

Model validation

To validate the model, the model was used to predict the plasma cholesterol concentrations of humans carrying genetic mutations, and these predictions were compared with experimental data obtained from literature. A genetic mutation was simulated by a model run with a case-specific set of parameters different from the normal situation, as follows. The rate constants for the reactions primarily affected by the given mutation were multiplied with a specific parameter ($f_{mut}$), according to Eq. 15, 16, or 17 (Table 2). This multiplication reflects the impact of the mutation on the reaction rate constant (i.e., fold reduction or increase). All other parameters were assumed to be unaffected by the given mutation. The values for $f_{mut}$ for each individual mutation were defined based on literature data as described in the Results section.
TABLE 2. Equations used in model development (Eq. 1–11), model calibration (Eq. 12–14), for simulation of the effect of human mutations (Eq. 15–17), and sensitivity analysis (Eq. 18)

| Differential Equations | Eq. |
|------------------------|-----|
| $\frac{d}{dt}(V_{\text{Liv}} \cdot [C]_{\text{Liv--FC}})$ | $v_1 + v_3 + v_{10} + v_{13} - v_{10} - v_{14} - v_{17} - v_{18}$ | Eq. 1 |
| $\frac{d}{dt}(V_{\text{Pla}} \cdot [C]_{\text{HDL--FC}})$ | $v_8 + v_{16} + v_{17} - v_9 - v_{13}$ | Eq. 2 |
| $\frac{d}{dt}(V_{\text{Pla}} \cdot [C]_{\text{non HDL--C}})$ | $v_9 - v_{10} - v_{21}$ | Eq. 3 |
| $\frac{d}{dt}(V_{\text{Pla}} \cdot [C]_{\text{non HDL--C}})$ | $v_6 + v_{11} - v_5 - v_7 + v_{21}$ | Eq. 4 |
| $\frac{d}{dt}(V_{\text{Per}} \cdot [C]_{\text{Per--C}})$ | $v_2 + v_7 - v_8 - v_{12}$ | Eq. 5 |
| $\frac{d}{dt}(V_{\text{Int}} \cdot [C]_{\text{Int--FC}})$ | $v_3 + v_4 + v_{14} - v_{20} - v_{16} - v_{15}$ | Eq. 6 |
| $\frac{d}{dt}(V_{\text{Liv}} \cdot [C]_{\text{Liv--CE}})$ | $v_{19} - v_6$ | Eq. 7 |
| $\frac{d}{dt}(V_{\text{Int}} \cdot [C]_{\text{Int--CE}})$ | $v_{20} - v_{11}$ | Eq. 8 |

Rate equations

$v_i = k_i^{ss} \cdot V$ (zero-order kinetics)  
$v_i = k_i^f \cdot V \cdot [C]$ (first-order kinetics)  
$v_{21} = k_{21}^s \cdot [C]_{\text{HDL--CE}} \cdot [C]_{\text{non HDL--C}} \cdot V$ (second-order kinetics)

Model calibration

$k_i^{ss} = \frac{v_i^{ss}}{V}$ (zero-order kinetics)  
$k_i^f = \frac{v_i^{ss}}{[C]^{ss} \cdot V}$ (first-order kinetics)  
$k_{21}^s = \frac{v_{21}^{ss}}{[C]_{\text{HDL--CE}}^{ss} \cdot [C]_{\text{non HDL--C}}^{ss} \cdot V}$ (second-order kinetics)

Mutation simulation

$k_{i,\text{mut},z} = f_{\text{mut}} \cdot k_i^z$ (zero-order kinetics)  
$k_{i,\text{mut},f} = f_{\text{mut}} \cdot k_i^f$ (first-order kinetics)  
$k_{21,\text{mut},s} = f_{\text{mut}} \cdot k_{21}^s$ (second-order kinetics)

Sensitivity analysis

$SC_{ij} = \frac{[C]_j^{ss} - [C]_j^k}{[C]_j^{ss}} \cdot \frac{k_i^k}{k_i^k - k_i^{ss,k}}$  

Model predictions were derived as follows: the differential equations (Eq. 1–8) were solved by numerical integration with the normal concentrations as initial values. Integration was performed using routine ode15s as implemented in MATLAB version 7.5 (R2007b) with the appropriate parameter value(s) for each subject (normal or mutant). The simulation was performed until steady state of all cholesterol pools in the model was achieved. Model predictions were defined as these steady-state concentrations.
Sensitivity analysis

To identify which reactions had a large influence on the eight predicted cholesterol pools in the model, a sensitivity analysis was performed. Percent changes in reaction rates were related to percent changes in tissue and plasma cholesterol pools. One by one, each kinetic constant was increased by 1% (leaving all other kinetic constants unchanged), and the model was used to predict the effect of this increase on all eight cholesterol pools that figured in the model. This analysis includes the response of all reactions in the model to the change in this kinetic constant. The effect of the increase in parameter of rate i on pool j was expressed in a sensitivity coefficient (SC) as defined in Eq. 18. The SC of the model was defined as the average of the SCs calculated with the eight submodels.

RESULTS

Conceptual model development

Fig. 1 presents the conceptual model for human plasma cholesterol levels. The model contains eight pools and 21 reactions, such as hepatic cholesterol synthesis (reaction 1), biliary cholesterol excretion (reaction 14), fecal cholesterol excretion (reaction 15), and CETP (reaction 21).

Model development and calibration

The human PBK model was formulated as differential equations (Eq. 1–8; Table 2) and rate equations (Eq. 9–11; Table 2) based on the conceptual model given in Fig. 1. The model was calibrated using compartmental volumes, steady-state cholesterol concentrations, and rates of cholesterol-involving reactions derived from literature. A detailed description of how data were derived, transformed into the correct units, and scaled to the 70 kg “reference man” as defined by the International Commission on Radiation Protection (19) can be found in the supplementary material. Concerning plasma cholesterol, the total plasma cholesterol concentrations were 5.25 mM for TC and 1.19 mM for HDL-C as was obtained from an inventory of data from 8809 US adults (22). Plasma cholesterol not present in the HDL-C pool (4.03 mM) was considered to be present in the non-HDL-C pool. The total HDL-C concentration (HDL-C, 1.19 mM) consists of HDL-FC and HDL-CE. The HDL-FC:HDL-CE ratio was 1:3 as obtained from Groener et al. (23). This ratio was applied to the HDL-C data above to obtain the HDL-FC and HDL-CE concentration, resulting in 0.30 mM for HDL-FC and 0.89 mM for HDL-CE. No distinction was made between non-HDL-free cholesterol and non-HDL-cholesterol ester (i.e., only the total [non-HDL-C] was considered).

A summary of the results is presented in Table 3. Several steady-state reaction rates were not directly obtained from data but instead were calculated from the other reaction rates using mass balances as indicated in Table 3 for $v^{95}$, $v^{10}$, $v^{10-12}$, $v^{17}$, and $v^{19-20}$.

The model predicted a steady state that matched all the data in Table 3, which indicates that the data, taken from various sources, were mutually consistent.

Model validation

As a model validation, 10 genetic variations known to affect cholesterol metabolism were simulated, and model predictions for TC, HDL-C, and non-HDL-C were compared with experimental data. The 10 mutations included mutations that cause familial hypercholesterolemia (FH), fish eye disease, Smith-Lemli-Opitz syndrome (SLOS), and other diseases. Details on all 10 mutations (numbered with roman digits) are given in Table 4 and are explained below. In the list of 10 mutations, two genes were included twice (APOB and LCAT), both as homozygote and as heterozygote variant.

Each mutation was simulated by multiplying the rate constant of the reaction affected with a specific parameter $f_{mut}$ defined for that mutation (see Eq. 15–17; Table 2). The parameter $f_{mut}$ is generally defined as the ratio of the value of a specific variable in carriers of the mutation to the value of that specific variable in controls. The specific affected variables for all mutations are given in Table 4. The parameter $f_{mut}$ for the simulation of a bile acid synthesis defect in the gene CYP7A1 (mutation X), for example, was defined as the ratio of the bile acid contents of the stools from carriers of the mutation to that in controls. The affected individuals had 5% of the amount of bile acids in their stools compared with healthy controls (24). The value of $f_{mut}$ was, therefore, set to 0.05 (Table 4). Values of the parameter $f_{mut}$ range from 0.00 for the SLOS mutation and the homozygote LCAT mutation to 0.65 for a variant of the CETP gene, implying that the list contained mutations that cause both mild and severe phenotypes.

Model predictions and experimental data are given in Fig. 2 (for TC), Fig. 3 (HDL-C), and Fig. 4 (non-HDL-C). Apart from LCAT deficiency (mutation VIII; Table 4), the model correctly predicted whether the TC, HDL-C, and non-HDL-C concentrations were decreased, increased, or relatively unchanged by the mutations. The average relative deviations between model predictions and experimental data were 36%, 49%, and 43% for TC, HDL-C, and non-HDL-C, respectively. This is considered successful within the present state-of-the-art of PBK modeling, where quantitative predictions may generally be correct within one order of magnitude (25–28). These model predictions are generally within the experimental error margin given the small patient groups sizes (generally n < 20).

Model analysis

An important step in modeling is model analysis, which is the step in which novel biological insight can be obtained. Model sensitivity analysis was performed to analyze which cholesterol concentrations were most affected by which biological reactions. Fig. 5 presents the sensitivity coefficients (SC) (Eq. 18) that express the sensitivity of the eight concentrations in the model toward changes in the kinetic parameters of each of the 21 reactions. A positive SC indicates that an increase in the reaction rate constant resulted in an increase of the predicted concentration. A negative SC indicates that an increase in the reaction rate constant resulted in a decrease of the predicted concentration. Some
concentrations responded to changes in many different rate constants (e.g., hepatic-free cholesterol [Liv-FC]), whereas other concentrations were sensitive to changes in only a few reactions. HDL-FC, for example, was only sensitive (SC < −0.25 or SC > 0.25) to changes in HDL-associated cholesterol esterification (reaction 9), and hepatic cholesterol esterification was only sensitive to HDL (reaction 17; Fig. 5). Fig. 5 indicates that some reactions strongly influenced many concentrations. The rate of hepatic uptake of cholesterol from non-HDL (reaction 5) influenced cholesterol esterification (reaction 9), and hepatic cholesterol transport was only sensitive to HDL (reaction 17; Fig. 5). Fig. 5 indicates that some reactions strongly influenced many concentrations. The rate of hepatic uptake of cholesterol from non-HDL (reaction 5) influenced

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Fig. 5 shows that Liver FC (Liv-FC) was not only sensitive to changes in the reactions that directly produced or consumed hepatic free cholesterol, such as biliary cholesterol excretion (reaction 14) and hepatic cholesterol catabolism (reaction 18), but also to peripheral cholesterol synthesis (reaction 2) and intestinal cholesterol esterification (reaction 20). Int-FC was primarily sensitive to changes in intestinal cholesterol esterification (reaction 20), biliary cholesterol excretion (reaction 14), and fecal cholesterol secretion (reaction 15).

Table 4. Description of the 10 human mutations used for model validation. The table includes the name of the gene carrying the mutation, the plasma cholesterol levels (TC, HDL-C, and non-HDL-C) of the subjects carrying the mutations (expressed as fold increase relative to the control group), the number of the reaction(s) affected by specific mutations, the severity of the affection expressed as $f_{mut}$ (Eq. 15–17), and the name of the variable used to determine $f_{mut}$. Reaction numbers correspond to numbers in Fig. 1.

| No. | Gene | TC (Relative to Control) | HDL-C (Relative to Control) | non-HDL-C (Relative to Control) | Reaction Affected | $f_{mut}$ | Experimentally Measured Variable Used To Determine $f_{mut}$ | Ref. |
|-----|------|-------------------------|-----------------------------|---------------------------------|------------------|---------|-------------------------------------------------|------|
| I   | LDLR | 1.85                    | 0.86                        | 2.17                            | 5, 7             | 0.38    | The fractional catabolic rate of APOB in LDL     | (61) |
| II  | APOB | 1.36                    | 0.85                        | 1.52                            | 5, 7             | 0.31    | The fractional catabolic rate of APOB in LDL     | (61) |
| III | APOB | 1.97                    | 1.12                        | 2.24                            | 5, 7             | 0.32    | The fractional catabolic rate of APOB in LDL     | (61) |
| IV  | ABCA1| 1.07                    | 0.22                        | 1.42                            | 8, 16, 17        | 0.41    | The cholesterol efflux rate to APOA1            | (67) |
| V   | APOE | 2.80                    | NA                          | NA                              | 5, 7             | 0.45    | The residence time of the carrier or control form of APOE in a normal subject | (68) |
| VI  | CETP | 1.01                    | 1.10                        | 0.98                            | 21               | 0.65    | The plasma level of CETP                         | (15) |
| VII | LCAT | 0.81                    | 0.79                        | 0.97                            | 9                | 0.62    | The in vitro determined activity of LCAT         | (46) |
| VIII| LCAT | 0.77                    | 0.19                        | 0.82                            | 9                | 0       | The in vitro determined activity of LCAT         | (46) |
| IX  | DHCR7| 0.20                    | NA                          | NA                              | 1, 2, 3          | 0.00    | The cholesterol synthesis activity in cultured fibroblasts | (69,70) |
| X   | CYP7A1| 1.74                    | 0.97                        | 2.09                            | 18               | 0.05    | The bile acid content of the stools              | (24) |

NA, not available

*Heterozygote familial hypercholesterolemia (FH).

**Heterozygote familial defective APOB (FDB).

***Homozygote familial defective APOB (FDB).

****Hypolipidoproteinemia.

*****Type III hyperlipoproteinemia.

******Heterozygote in exon 15.

*******Heterozygote familial LCAT deficiency (incl. Fish eye disease).

********Homozygote familial LCAT deficiency (incl. Fish eye disease).

*********Smith-Lemli-Opitz syndrome (SLOS).

**********Bile acid synthesis defect.

Fig. 2. PBK model predictions compared with experimental data for TC concentrations (relative to control) for 10 different human mutations. Mutation numbers correspond to the numbers in Table 4.
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Of special interest for the purpose of the present model are the SC values for cholesterol concentrations in plasma (TC, HDL-C, and non-HDL-C) that are correlated with the risk for coronary heart disease (10). Sensitivity coefficients for the influence of the different reactions on these concentrations are given in Fig. 6.

Hepatic uptake of cholesterol from non-HDL (reaction 5), hepatic transport of cholesterol to HDL (reaction 17), and hepatic cholesterol esterification (reaction 19) were the reactions that showed the largest influence on TC (i.e., resulting from the combined influence of these reactions on HDL-C [Fig. 6, middle panel] and non-HDL-C [Fig. 6, right panel]). Reactions 6, 11, 16, and 21 had the smallest effect on TC. The HDL-C concentration (Fig. 6, middle panel) was mainly dependent on hepatic transport of cholesterol to HDL (reaction 17), CE transfer from HDL to non-HDL (reaction 21), and hepatic uptake of cholesterol from non-HDL (reaction 5). The model predicted (Fig. 6, right panel) that non-HDL-C was highly dependent on hepatic uptake of cholesterol from non-HDL (reaction 5) and mainly on hepatic transport of cholesterol to HDL (reaction 17) and hepatic cholesterol esterification (reaction 19).

In general, as seen in Fig. 6, sensitivity coefficients for TC were more similar to those for non-HDL-C than to those for HDL-C because only a small fraction of plasma cholesterol is present in HDL-C.

Finally, this sensitivity analysis revealed that, according to the model, there are several efficient ways to lower non-HDL-C concentrations and increase HDL-C concentrations simultaneously by modulating only one single reaction (Fig. 6, middle and right panels). The most potent reactions

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**Fig. 3.** PBK model predictions compared with experimental data for HDL-C (relative to control) for 10 different human mutations. Mutation numbers correspond to the numbers in Table 4. NA, no data available.

**Fig. 4.** PBK model predictions compared with experimental data for non-HDL-C (relative to control) for 10 different human mutations. Mutation numbers correspond to the numbers in Table 4. NA, no data available.
genetic, nutritional, and pharmaceutical effects on plasma cholesterol levels.

As all models, our model is a compromise between simplicity and complexity (37). A too simple model is not useful to simulate multiple interventions because such a model will lack the targets of at least part of these interventions. A too complex model is not useful either because insufficient experimental data will be present to define the parameters (calibration) or to validate the model.

One of the important necessary simplifications made was to restrict the description of lipoprotein metabolism to cholesterol alone. As a consequence, no distinction was made between different fractions of non-HDL-C (i.e., LDL, IDL, and VLDL) in the present model. Including a more detailed mechanistic description of lipoprotein metabolism is a highly complex task that will necessitate, for example, to include the link between cholesterol metabolism and triglyceride metabolism. Although significant progress in this field is being made (4, 38, 39), this was considered too ambitious for the present stage of model development.

As a consequence of these necessary simplifications, rates derived from model-based interpretation of stable isotope tracer data were unsuited for model calibration because the structure of well-established isotopic tracer kinetic models (33 and references therein) did not match with the present model due to different aggregations of reactions. A factor further hampering the use of these data is that the tracer analysis considers bidirectionality of fluxes, whereas our PBK model formulation had to be limited to unidirectional (i.e., net) fluxes to keep the number of parameters at a minimum. As a result, using the isotopic flux data for calibration of the PBK model was not possible.

The following two examples illustrate some of the difficulties arising from the different structures of the present PBK model vs. the established tracer kinetic models described in Schwartz et al. (33): (1) The tracer kinetic model assumes all cholesterol to be synthesized in the liver, whereas in the present PBK model the liver contributed only 10% of total cholesterol synthesis, in line with data from Dietschy and Turley (40). (2) Schwartz et al. (33) reported that the liver does not take up HDL-CE, whereas the corresponding reaction (reaction 10) in the PBK model carries a considerable flux (2.93 mmol/man/day),

**DISCUSSION**

In silico models have been used for various purposes in the study of cholesterol metabolism, such as in the interpretation of isotope-labeling studies (4–6, 33), in the analysis of the regulatory pathway of cholesterol synthesis (34), or in making predictions of the effect of genetic mutations or food and drug interventions (35, 36). These models, however, could predict the effect of a few genetic mutations only. The aim of this study was to develop a model that can be applied in the prediction of a wide variety of genetic, nutritional, and pharmaceutical effects on plasma cholesterol levels.

As all models, our model is a compromise between simplicity and complexity (37). A too simple model is not useful to simulate multiple interventions because such a model will lack the targets of at least part of these interventions. A too complex model is not useful either because insufficient experimental data will be present to define the parameters (calibration) or to validate the model.

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As a consequence of these necessary simplifications, rates derived from model-based interpretation of stable isotope tracer data were unsuited for model calibration because the structure of well-established isotopic tracer kinetic models (33 and references therein) did not match with the present model due to different aggregations of reactions. A factor further hampering the use of these data is that the tracer analysis considers bidirectionality of fluxes, whereas our PBK model formulation had to be limited to unidirectional (i.e., net) fluxes to keep the number of parameters at a minimum. As a result, using the isotopic flux data for calibration of the PBK model was not possible.

The following two examples illustrate some of the difficulties arising from the different structures of the present PBK model vs. the established tracer kinetic models described in Schwartz et al. (33): (1) The tracer kinetic model assumes all cholesterol to be synthesized in the liver, whereas in the present PBK model the liver contributed only 10% of total cholesterol synthesis, in line with data from Dietschy and Turley (40). (2) Schwartz et al. (33) reported that the liver does not take up HDL-CE, whereas the corresponding reaction (reaction 10) in the PBK model carries a considerable flux (2.93 mmol/man/day),

![Fig. 5. Color representation of the sensitivity coefficients that quantify the influence of changes in the rate constants of the different reactions on the different concentrations. See text for details. Reaction numbers correspond to the numbers in Figure 1.](image)

![Fig. 6. Sensitivity coefficients (see Eq. 18) for the different reactions toward TC (left panel), HDL-C (middle panel), and non-HDL-C (right panel). Reaction numbers correspond to the numbers in Figure 1.](image)
which is more in line with the statement of Rinninger et al. (41) based on data obtained with primary hepatocytes (i.e., “high-density lipoprotein cholesteryl esters were selectively taken up by hepatocytes and are hydrolyzed independently from the classical lysosomal catabolic pathway”). Thus, although the two models differ markedly in structure, supporting evidence for the PBK model structure can also be found in the literature. This suggests that valuable insights could be obtained from future work that would analyze structure variations in both models while taking calibration data for both models into account. For instance, one could investigate whether directing $v_{5}$ and $v_{19}$ into liver CE and adding a pathway that is the reverse of $v_{19}$ to represent lysosomal hydrolyses is equivalent (in terms of outcomes and conclusions) to directing them into the liver FC pool as in the present PBK model. Likewise, different alternative structures of the tracer kinetic models may be investigated that are more in line with the PBK model. Such models could, for example, incorporate constraints on net fluxes derived from the PBK model in the isotopic tracer data analysis.

The level of complexity of the model described here was apparently an acceptable compromise because the model could successfully be calibrated from literature data by flux balancing with only a limited set of assumptions (see supplementary information). Nevertheless, the consequences of the implemented calibration procedure may be a point for further attention. Data used were from a variety of different unconnected studies that used different experimental protocols and methods, often applied to very small groups of subjects of different weight and age. This carries the inherent risk of data inconsistencies and potentially large influences of individual variation. Although this risk was mitigated as much as possible by scaling all data to a 70 kg reference man, questions may remain as to the impact of the calibration on the predictions by the model. The sensitivity analysis gives some insight in this issue (i.e., from Fig. 6 it appears that variation of reactions 6, 11, and 16 will have little influence on the plasma cholesterol predictions of the model). Although a full variability analysis would be required to cover this issue, this was not feasible because data are too sparse to give a statistically meaningful estimate and to validate the analysis, but we generally saw a 25% variation across any dataset. Rather, we discuss in the following sections some specific results that seem at variance with established cholesterol flux analysis literature (33 and references therein). Peripheral cholesterol loss (reaction 12) was 2.62 mmol/man/day, which is comparable to the sum of net bile acid loss (1.03 mmol/man/day) and net fecal cholesterol loss (1.85 mmol/man/day), which are generally considered the main routes for cholesterol loss from the body. Beyond skin sloughing and steroid hormone production, one could ask which candidate processes could carry this flux. Because the estimation of total cholesterol synthesis is based on in vivo squalene isotopic labeling data, we do not consider a mistake in this estimation as a possible explanation for the missing peripheral cholesterol excretion. Rather, we hypothesize that sebum production might contribute considerably in this regard. Human sebum secretion rates from forehead skin are reported to lie between 1.3 and 3.3 mg/10 cm$^2$/3 h (42). NMR studies indicate that the molar fractions of CE and squalene in sebum are 0.03 and 0.28, respectively, with the other constituents being triglycerides, fatty acids, and wax esters (43). With a total body area of approximately 2 m$^2$, taking into account the molar weights of the different constituents and assuming uniform secretion rates, an upper bound for the total CE and squalene flux may be as high as 2.5 mmol/man/day. Although this estimation seems to support the possibility that peripheral cholesterol loss may be as high as the model calibration suggests, independent validation experiments are necessary to confirm the hypothesis that sebum excretion is a possible explanation for the missing peripheral cholesterol excretion.

Another item is the transfer of FC from liver to HDL ($v_{17}$, 6.91 mmol/man/day), which in the present analysis greatly exceeded total hepatic uptake of HDL-FC and HDL-CE combined ($v_{19} + v_{13}$, 4.50 mmol/man/day), whereas in Schwartz et al. (33) the reverse was observed (i.e., more liver uptake of HDL-C than transfer from liver to HDL). However, in Schwartz et al. (33), this net flux is the difference between very high fluxes (>46 mmol/man/day) that operate in both directions. Thus, both models indicate a large contribution to plasma HDL coming from the liver. This has also been observed in animal studies and in our previous mouse work (12).

These observations indicate that, although the flux distribution in our model deviated strongly from that seen in established isotope tracer studies for reasons discussed above, supporting evidence for the PBK model structure and flux predictions can be found in the literature.

The key question is whether the model is valid for its intended use to predict changes in plasma cholesterol concentrations following different dietary regimens, pharmacological treatments, or genetic variation. Indeed, the PBK model was able to predict the effects of 10 human mutations (Figs. 2–4), including a mutation in the LDLR gene (mutation I, responsible for FH) and the DHCR7 gene (mutations IX, responsible for SLOS). While simulating this latter syndrome, negative concentrations were predicted for several submodels. This must be due to the fact that these submodels retain zero-order kinetics (i.e., saturated Michaelis-Menten kinetics) when under the extreme conditions associated with blocked cholesterol synthesis in SLOS (44), cholesterol concentrations are lowered to such an extent that first-order kinetics would be more appropriate.

Model predictions for TC deviated on average less than 40% from experimentally observed values, which is relatively good compared with the current state of the art for PBK models of exogenous substances (26–28, 45). This is all the more remarkable because the model was obtained via a relatively straightforward translational adaptation of our previously developed mouse model.

The only case where a large deviation between model predictions and experimental data (HDL-C concentration) were seen is in LCAT deficiency: mutation VIII. The model
predicted an increase in HDL-C, whereas a decrease is observed in reality (Fig. 3). This increase is in the form of HDL-FC and not in HDL-CE (data not shown). The resulting shift in CE/FC ratio predicted by the model is in fact similar, as seen in the literature (46). A possible explanation for the deviation between model predictions and experimental data is that, in reality, a maximum of HDL-FC might exist. If this maximum is reached, the transport of free cholesterol to HDL (reactions 8, 16, and 17) will be inhibited, thereby causing HDL-C lowering. The model in its present form does not take this into account.

As shown in Fig. 6, the model predicted that an increased dietary intake of cholesterol (reaction 4) will lead to an increased TC level, an increased non-HDL-C level, and a slightly lowered HDL-C level. This is in agreement with findings in nutritional studies (see meta-analysis in Ref. 47). The model also predicted that non-HDL-C is mostly affected by hepatic cholesterol esterification (reaction 19) and hepatic uptake of cholesterol from LDL (reaction 5). This confirms that the liver is a dominant organ in determining the plasma cholesterol levels (13).

A decrease in hepatic cholesterol synthesis (reaction 1) resulted in a decrease of the non-HDL-C (Fig. 6, right panel) and in an increase of HDL-C. This is in agreement with the outcome of statin-mediated inhibition of hepatic cholesterol synthesis (48). In reality, statin therapy will also cause an up-regulation of the LDLR and thereby the activity of hepatic non-HDL-C uptake, increasing the non-HDL-C lowering effect.

A decrease in the activity of CETP (reaction 21) had a larger relative effect on HDL-C than on non-HDL-C (Fig. 6). This is in agreement with the outcome of torcetrapib-mediated CETP inhibition (49) and CETP mutations (mutation VI, Table 4). Taken together, these findings illustrate that the described model can indeed be helpful to predict effects of dietary and pharmaceutical interventions.

A recent genome-wide association study (GWAS) has found 95 SNPs that correlated with altered TC, HDL-C, LDL-C or triglycerides concentrations (50). At least 19 of these SNPs were near genes that are involved in one or more of the reactions in the model. The gene ABCA1, for example, is involved in the transport of cholesterol to HDL (reactions 8, 16, and 17).

We compared effect sizes of the 19 SNPs given by Teslovich et al. (50) with the sensitivity coefficients of associated reactions (Fig. 6) by Spearman correlation and found a positive correlation between our findings and the ones reported by Teslovich et al. (50) for HDL-C, LDL-C, and TC (all $P < 0.05$) (data not shown). This is an additional validation of the present model and implies that the developed model is useful to study the implications of genetic variations on cholesterol metabolism.

The GWAS study (50) also reports several SNPs correlating with cholesterol concentrations in plasma near genes that could not be directly linked to a specific reaction in our model (Fig. 1), like in HNF4A, CILP2, and ANGPTL3. This absence of a direct link is the result of essential simplifications needed to construct the model.

We conclude that the approach of first developing a computational PBK model for the mouse and then translating it into a human model as described in this paper resulted in an accurate model for the prediction of plasma cholesterol concentrations in humans. Sensitivity coefficients derived from the model correlated well with recent independent GWAS data on plasma cholesterol. Because the model correctly predicted key features of the effect of increased dietary cholesterol intake and of statin treatment and CETP inhibition, we expect that the model can also be used to predict the effects of a wider variety of pharmacological and dietary interventions on plasma cholesterol levels in humans, which will be the subject of further work.

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