In silico modeling of the dynamics of low density lipoprotein composition via a single plasma sample

Martin Jansen,1,* Peter Pfaffelhuber,† Michael M. Hoffmann,* Gerhard Puetz,* and Karl Winkler*

Institute of Clinical Chemistry and Laboratory Medicine,* Medical Center, and Department of Mathematical Stochastics,† University of Freiburg, Germany

Abstract Lipoproteins play a key role in the development of CVD, but the dynamics of lipoprotein metabolism are difficult to address experimentally. This article describes a novel two-step combined in vitro and in silico approach that enables the estimation of key reactions in lipoprotein metabolism using just one blood sample. Lipoproteins were isolated by ultracentrifugation from fasting plasma stored at 4°C. Plasma incubated at 37°C is no longer in a steady state, and changes in composition may be determined. From these changes, we estimated rates for reactions like LCAT (56.3 µM/h), β-LCAT (15.62 µM/h), and cholesteryl ester (CE) transfer protein-mediated flux of CE from HDL to LDL/VLDL (21.5 µM/h) based on data from 15 healthy individuals. In a second step, we estimated LDL’s HL activity (3.19 pools/day) and, for the very first time, selective CE efflux from LDL (8.39 µM/h) by relying on the previously derived reaction rates. The estimated metabolic rates were then confirmed in an independent group (n = 10).

Due to the high variability of lipid and apolipoprotein composition, lipoproteins form a heterogeneous set of particles characterized by different features and metabolic fate. Lipoproteins are classified according to their density. In the healthy fasting state, established classes of lipoproteins are VLDLs, IDLs, LDLs, and HDLs (2), which can be further subdivided into lipoprotein subfractions.

Not only the concentration of LDL cholesterol but also the particle size/density distribution in LDL is associated with CVDs. LDL can be differentiated into large-buoyant and small-dense LDL (lb-LDL and sd-LDL, respectively). LDL subtype patterns with predominantly sd-LDL (3) or with predominantly lb-LDL (4) are associated with an increased risk for CVDs.

Although the clinical assessment of lipoprotein metabolism and risk prediction are based on simple measurements of steady-state concentrations like LDL- and HDL-cholesterol, lipoprotein metabolism is a dynamic transport process of lipids. In fact, most of the potential disease-causing effects of lipoproteins may lie yet unresolved in the transient nature of lipoprotein metabolism and lipid fluxes.

LDL is primarily cleared from plasma via holoparticle uptake mediated by the LDL receptor, which requires apolipoprotein B-100 (ApoB) as a ligand. The exchange of TG and CE between lipoprotein particles is predominantly facilitated by the enzyme cholesteryl ester transfer protein (CETP). There are other enzymes and receptors besides CETP affecting LDL. Particles may lose TG and PL by the action of lipases. FC is converted to CE by the action of LCAT (5), and CE can be removed by selective CE efflux (6–9).

Different enzymes hydrolyze TG in lipoproteins in plasma. The most important are LPL and HL. While LPL...
acts on large particles such as VLDL and chylomicrons, 
HL is important for TG hydrolysis in LDL and HDL. How-
ever, as the ranges of their specificities are not clearly di-
istinguished, both HL and LPL might act on lb-LDL (10).

The role of CE efflux in particles containing ApoB has 
not been clarified. There is little published data dealing 
with this reaction occurring either as selective CE efflux 
via lipoprotein binding sites or as transfer of CE to HDL 
during lipolysis of VLDL (9). It is assumed that the selec-
tive CE efflux is effective for all ApoB-containing particles 
but may not play such a significant role as in HDL metabo-
lism (11).

LCAT’s main activity is found in HDL (12). However, it 
is also active in LDL to some extent. This LCAT activity 
is referred to as LCAT β-activity (5).

The exact mechanism of lipid transport via CETP is still 
unclear. It is assumed that CETP activity is proportional 
to the particle surface area (13). Qiu et al. (14) suggest that 
it mediates an equimolar exchange, not a transfer of lip-
ids. Neither TG nor CE is preferred by CETP. Hence, 
CETP action leads to equilibration of TG and CE between 
lipoproteins and does not mediate a directed transfer of 
one lipid species against a concentration gradient.

There is also a transfer of FC from erythrocytes to lipo-
proteins and among lipoproteins themselves. Lange et al. 
(15) suggest that FC moves between plasma and erythro-
cytes via aqueous diffusion. According to Estronca et al. 
(16), who examined the passive transport of FC in blood, 
there is a relatively fast passive exchange of FC among lip-
proteins (and between lipoproteins and albumin) and a 
much slower exchange of FC between lipoproteins and 
erthrocytes. They suggest that FC associated with lipopro-
etins in vivo is equilibrated after 15 min among all lipopro-
fraction. In contrast, it takes 400–600 min in vivo for 
lipoprotein and erythrocyte FC to equilibrate. The FC-
consuming LCAT reaction causes an FC gradient among 
lipoproteins, especially HDL to other lipoproteins, and 
between lipoproteins and membranes of endothelial or 
blood cells. Consequently, passive FC transport leads to a 
net flux of FC from the cellular compartment to lipopro-
etins, especially to the HDL fraction.

While steady-state concentrations of key lipoprotein 
constituents are easily accessible, the dynamic processes 
underlying lipoprotein metabolism are becoming ever 
more important. The established method to gather infor-
mation about the dynamics of lipoproteins or its compo-
nents is to label protein or lipids endogenously, using 
radioactive or stable, nonradioactive isotopes. These data 
are then evaluated using (multi-) compartmental model-
ing based on sets of differential equations (17–21). Be-

side this tracer kinetic approach, there are other less 
common approaches to model certain aspects of the 
dynamics of lipoprotein metabolism (22–25).

Besides elaborate isolation and measurement of lipo-
protein fractions, the tracer kinetic approach involves 
labeling of lipids or amino acids with isotopic tracer, 
multiple blood sampling over a long period of time (espe-
cially if LDL is considered due to its longevity), and the 
measurement of key compounds by mass spectrometry.

Here we present a novel two-step combined in vitro and 
in silico approach that enables the estimation of key reac-
tions such as the transport of neutral lipids via CETP, TG 
hydrolysis via HL, and esterification of cholesterol via 
LCAT via a single blood sample.

MATERIALS AND METHODS

Human plasma samples

We examined fasting plasma from 9 females and 16 males 
(aged 21 to 50 years) of whom 4 females and 6 males were 
assigned to a validation group without lipoprotein-metabolism dis-
order. Informed consent was obtained from each volunteer, and 
the trial was approved by the Ethics Review Committee of the 
University of Freiburg.

Plasma was separated at 4°C and siphoned in test tubes. Glass 
tubes were used to avoid FC and PL interaction with plastic sur-
faces. Two glass tubes were kept for 1 h at 37°C, while the other 
two glass tubes were stored at 4°C. The lipid parameters TG, FC, 
PL, and total cholesterol were determined enzymatically with 
PAP-Methods (DiaSys; Diagnostic Systems GmbH, Holzheim, 
Germany). The difference between total cholesterol and FC was 
interpreted as CE. The parameters ApoA1 and ApoB were deter-
ned using turbidimetric methods (Greiner Bio-One GmbH, 
Frickenhausen, Germany). All parameters were determined with 
an Olympus A4 640 (Olympus Diagnostica Lab Automation, Um-
kirch, Germany).

Separation of lipoprotein fractions

All samples were stored at 4°C during preparation. Each sam-
ple’s lipoprotein profile was determined by preparative ultracen-
trifugation as previously described (23). LDL was separated into 
six subfractions, namely LDL1, LDL2, LDL3, LDL4, LDL5, and 
LDL6 [LDL5 and LDL6 are also referred to as sd-LDL (26)]. 
HDL was separated into three subfractions HDL2b, HDL2a, 
and HDL3. Below, the subfractions LDLI (LDL1 and LDL2), 
LDLL (LDL3 and LDL4), and LDLIII (LDL5 and LDL6) are 
considered. The fraction with density >1.210 g/ml is referred to 
as lipoprotein deficient serum (LDS). In the isolated fractions of 
VLDL, IDL, LDL, HDL, and LDS, as well as in the LDL and HDL 
subfractions, TG, CE, FC, PL, ApoB, and ApoA1 concentrations 
were determined. The results of the corresponding two samples 
per experimental arm were averaged.

Modeling CETP action

CETP action applied in the model is similar as done by Potter 
et al. (27). A shuttle model (28) was assumed, and equimolar 
hoehereexchange of TG and CE between CETP and lipoproteins 
was modeled.

CETP is loaded with either CE or TG. CETP does not prefer 
TG to CE. Hence, the probability of a heteroexchange (TG for 
CE exchange or vice versa) given a TG-associated (or CE-associated) 
CETP that reacts with an LDL particle loaded with Lce, CE and 
Ltg, TG molecules is obtained by the \( \frac{L_{\text{ce}}}{(L_{\text{ce}} + L_{\text{tg}})} \) or \( \frac{L_{\text{tg}}}{(L_{\text{ce}} + L_{\text{tg}})} \) ratio of the particle.

The parameter \( \text{CETPTG} \)

The model-parameter \( \text{CETPTG} \) denotes the ratio of CETP oc-
cupied by TG to total CETP in plasma. Let \( P \), denote the mean 
olipoprotein in the \( i \)th fraction regarding the TG, FC, and CE 
composition, where \( i \in \{ \text{VLDL}, \text{IDL}, \text{LDL-I}, \text{LDL-II}, \text{LDL-III}, \text{HDL2b}, \text{HDL2a}, \text{HDL3} \} \). Let \( s \), \( k \), and \( q \) denote functions allocating
the surface, the concentration of particles, and the molar \(\text{TG}/(\text{CE} + \text{TG})\) ratio with respect to \(P_s\).

Assuming the features of CETP described in the introduction, set

\[\text{CETP}_{\text{TG}} = \sum_{i \in I} s(P_i) q(P_i) k(P_i) / \sum_{i \in I} s(P_i) q(P_i) \]

The number of ApoA1 per HDL particle in the three HDL subfractions is necessary to calculate the concentration of HDL particles and their surface. This was gleaned from Verder et al. (29), who estimate that there are 4.5, 3.7, and 3.4 ApoA1 per HDL2b, HDL2a, and HDL3, respectively. Using the number of TGs and CEs per particle, we computed the surface of a lipoprotein particle by assuming that the lipoprotein core consists only of TG and CE and that the outer shell is 2.02 nm thick (30).

**Density computation**

An accurate density determination of the simulated particles in the LDL fraction is crucial to our model. We computed this density under the assumption that there is no protein other than ApoB in this lipoprotein. Molar weights and volumes of FC, PL, and TG, as well as the weights of CE and ApoB, were adopted from Teerlink et al. (31). The molecular number of PLs per particle, which is necessary to compute the density, was estimated by the linear regression dependent on CE and FC per particle:

\[
p_{\text{reg}} \left( \frac{\text{CE}}{\text{ApoB}} , \frac{\text{FC}}{\text{ApoB}} \right) = \frac{m_{\text{CE}}}{m_{\text{ApoB}}} \cdot \frac{\text{CE}}{\text{ApoB}} + \frac{m_{\text{FC}}}{m_{\text{ApoB}}} \cdot \frac{\text{FC}}{\text{ApoB}} + b
\]

Here CE/ApoB and FC/ApoB denote the molecular number of CE and FC per particle. The molecular volume of CE and ApoB was estimated by minimizing the distance between the expected mean density and corresponding computed density in the LDL subfractions given the molecular masses and volumes mentioned above.

**Modeling LDL dynamics**

We modeled the temporal change in an in silico LDL particle characterized by its TG, CE, and FC content. Let \(T(t) > 0\), \(C(t) > 0\), and \(F(t) > 0\) be the expected numbers of TG, CE, and FC molecules, respectively, at time \(t\), and \(t_0\) the time when the simulation starts. The triple \((T(t_0), C(t_0), F(t_0))\) at the beginning of the simulation is hereafter referred to as the starting point.

Table 1 illustrates the reactions we modeled. While all reactions occur in vivo, only some of the reactions occur in vitro.

Let \(d_t > d(T(t), C(t), F(t))\) denote the density of the LDL particle considered at time \(t\). The function \(g(T(t), C(t), F(t))\) is defined as follows:

\[
g(T(t), C(t), F(t)) = \begin{cases} 1 & \text{if } d_t \leq 1.019 \\ 0 & \text{if } d_t \geq 1.063 \\ -d_t - 1.063 & \text{else} \end{cases}
\]

The numbers 1.019 and 1.063 refer to the boundaries in g/ml of the LDL density range and \(0.044 = 1.063 - 1.019\).

The reactions in the model do not underlie mass-action kinetics. Given starting point \((T(t_0), C(t_0), F(t_0))\) at time \(t_0\), we consider the following system of autonomous differential equations for reaction \(R_i\) corresponding to the removal of a TG, CE, or FC per particle:

\[
\frac{dT(t)}{dt} = -r_1 T(t) + r_2 C(t) - r_3 F(t)
\]

\[
\frac{dC(t)}{dt} = -r_4 C(t) + r_5 F(t)
\]

\[
\frac{dF(t)}{dt} = r_6 - r_7 \left( \frac{C(t)}{F(t)} \right)
\]

Here for \(i = 1, \ldots, 7\), \(r_i\) denotes the reaction rate of the corresponding reaction \(R_i\). Thus, \(r_1\), \(r_2\), \(r_3\), and \(r_4\) are the rates for the removal of a TG, CE, exchange of an FC for a CE, and removal of an FC, respectively. The \(R_6\) reaction describes the influx of FC in the LDL fraction per particle.

The function \(g(T(t), C(t), F(t))\) causes a linear decrease in reaction \(R_6\) depending on the density of the simulated particle, when this particle is in the LDL density range.

Let \(r_{\text{cetp}}\) be the CETP-association rate per particle. Set

\[
r_{\text{cetp}} = r_{\text{cetp}} \cdot \text{CETP}_{\text{TG}} \quad \text{and} \quad r_{\text{cetp}} = r_{\text{cetp}} \cdot (1 - \text{CETP}_{\text{TG}})
\]

The velocities of the reactions consuming CE or TG are weighted by the molar proportion of CE or TG to the sum of TG and CE, respectively, as these hydrophobic lipids compete for space, especially during the CETP reaction. The velocities of the LCAT reaction \(R_6\) and reaction \(R_7\) are proportional to the particles FC/(FC + PL) ratio, to model the properties of the lipoprotein monolayer.

**Table 1. Reactions of the LDL model and their properties**

| Reaction | Chemical Equation | Metabolic Process | Reaction Rate | Reactions Taking Place In Vitro<sup>a</sup> |
|----------|------------------|------------------|--------------|---------------------------------|
| \(R_1\)  | \(TG \rightarrow \emptyset\) | HL               | \(r_1\)      |                                |
| \(R_2\)  | \(CE \rightarrow \emptyset\) | Selective CE efflux | \(r_2\)     |                                |
| \(R_3\)  | \(CE \rightarrow TG\) | CETP             | \(r_{\text{cetp}} \cdot \text{CETP}_{\text{TG}}\) | +                |
| \(R_4\)  | \(TG \rightarrow CE\) | CETP             | \(r_{\text{cetp}} \cdot (1 - \text{CETP}_{\text{TG}})\) | +                |
| \(R_5\)  | \(FC \rightarrow CE\) | LCAT             | \(r_6\)      |                                |
| \(R_6\)  | \(\emptyset \rightarrow FC\) | FC influx        | \(r_7\)      |                                |
| \(R_7\)  | \(FC \rightarrow \emptyset\) | FC efflux        | \(r_8\)      |                                |

<sup>a</sup>During storage of plasma for 1 h at 37°C.
In an LDL particle, FC can be lost due to direct LCAT action (reaction $R_5$). Moreover, we assume that the passive redistribution among lipoproteins of FC in vitro causes a net loss of FC in LDL (reaction $R_7$).

The reaction $R_6$ describes the influx of FC into the LDL fraction, which does not occur during in vitro storage of plasma. The sources of FC entering the LDL fraction are assumed to be mainly erythrocytes (16).

We also assumed that no non-LDL lipoproteins enter the LDL density range after 1 h of storage at 37°C.

To model the particle uptake in the in vivo situation, the lifetime of each particle was assumed to be exponentially distributed with parameter $\lambda$, so the density $\lambda e^{-\lambda t}$ can be allocated to each $t (T(t), C(t), F(t))$ starting at $t_0 = 0$. The parameter $\lambda$ was adopted from the review by Marsh et al. (32) and set to 0.46/24 pools/h. Taken together, the mean concentration and mean composition for each $t \geq t_0$ can be computed by solving the system numerically.

Parameter inference

There are several in vivo reactions affecting lipoproteins and maintaining their composition in a steady state. When using only data from a single blood withdrawal and under the assumption that the lipoprotein system is in steady state, we gather too few data points and no information about the temporal dimension. No reasonable inference of reaction rates is possible. When plasma is kept at 37°C in vitro, the steady state is no longer given, as only some of the reactions take place (Table 1).

Based on the dynamic changes measured in vitro, these reactions can be computed by parameter inference. Having that information at hand, the dynamics of the other reactions not occurring in vitro can be inferred by adding steady-state compositional data.

The concept of parameter inference via two steps combining the steady state and in vitro dynamic data is illustrated in Fig. 1.

In detail: First, the model was fitted by the change in lipoprotein composition after 1 h of plasma storage in vitro at 37°C. Thereby only the parameters $r_6$, $r_7$, and $r_{cetp}$ were fitted, as in this situation all other reaction rates were assumed to be zero. The parameter $\text{CETP}_{TG}$ was directly derived from the corresponding experimental data. The parameter $r_{cetp}$ was fitted by considering the change in TG per ApoB in the LDL fraction after 1 h of storage at 37°C. Given $r_{cetp}$ and $\text{CETP}_{TG}$ we estimated the flux of CE and TG into and out of the fractions VLDL, IDL, LDL, and HDL. The amount of CE generated in each fraction of these four due to the LCAT reaction was estimated by comparing the change observed in CE in these fractions after 1 h of storage at 37°C with the expected change in CE due to CETP action. Moreover, the loss of FC due to reaction $R_7$ in the LDL fraction after 1 h of plasma storage at 37°C was estimated.

The parameters derived in this first step were fixed for the second step of parameter inference. Here, the LDL composition of the plasma stored at 4°C before lipoprotein separation was assumed to represent an in vivo steady state, and the remaining parameters $r_1$, $r_3$, and $r_4$ were inferred.

Given our experimental data, the quality of a corresponding simulated profile was expressed by the sum of the least square distances of the ApoB concentration and the CE, FC, and TG lipoprotein components. Each of these four classes was weighted by the reciprocal of the corresponding mean value of the ApoB concentration or number of lipids per ApoB at baseline, respectively. Thus, each class contributes more or less equally to the sum of the least squares.

All optimizations were done using several runs of the downhill simplex algorithm (33). All simulations and nonstatistical computations were performed by Scilab 5.4.0 (Scilab Enterprises, Le Chesnay, France).

Statistics

The changes in CE, FC, TG, PL, ApoB, and ApoA1 in lipoprotein (sub-)fractions and plasma were tested for significance using the Wilcoxon signed-rank test. All statistical analyses were done using IBM SPSS version 21.0 (IBM SPSS Statistics; IBM Corporation, Chicago, IL).

RESULTS

Based on experimental data, we captured the characteristics of ApoB, TG, CE, and FC of normolipidemic volunteers in three LDL subfractions using a mathematical model (Table 1). The following parameters are determined in two steps: $r_1$, $r_2$, $r_5$, $r_6$, and the FC, CE, and TG load of the LDL starting point. The model computes the time evolution of the mean CE, TG, and FC molecule number of a LDL particle.

Effect of plasma storage at 37°C for 1 h

Table 2 illustrates the molecular number of VLDL, IDL, and LDL (subfractions) components and the ApoB concentration of plasma stored for 1 h at 4°C, which is regarded as baseline, or 37°C. There is a significant decrease in FC and a significant increase in TG in each LDL subfraction. Furthermore, ApoB mass increases significantly in LDL-II. In the VLDL fraction, there is a significant increase in CE molecules per particle of 264 ± 96 molecules and a significant decrease in TG molecules per particle of 164 ± 203 molecules, respectively.

The change in lipoprotein components in HDL (subfractions), LDS, and total plasma is shown in Table 3. While ApoA1 decreases significantly in the HDL3 fraction, it increases significantly in the other HDL subfractions. In total, plasma storage for 1 h at 37°C causes a significant increase in CE of 82.0 μM and a significant decrease in FC of 55.1 μM. In the LDL and HDL fraction, 27.6 and 10.3 μM FC are lost, while 9.3 and 29.0 μM CE are acquired, respectively. PL is lost in VLDL, IDL, LDL, and HDL. A corresponding amount is gained in LDS. The loss of PL in VLDL was described by Yen et al. (12), who proposed that it is mediated by CETP.

Model configuration

The parameters of the regression for the number of PL molecules per LDL particle are $m_{PL} = 1.075 \times 0.222$, $m_{PL} = 1.075 \times 0.431$, and $b = 1.075 \times 122.33$. The factor 1.075 is motivated by the fact that ~7.5% of PLs are not measured, using the above-described method of PL measurement (31). The coefficient of determination of the regression is 0.814.

Optimization of CE and ApoB volume using the LDL density ranges, given the LDL lipid data at 4°C storage and the molecular masses and volumes described in Materials and Methods section, delivers a molecular volume of 674.79 ml/mol for CE and 371,511.2 ml/mol for ApoB.
Parameter inference, step 1

Given the parameter $CETP_{TG} = 0.163$, optimization resulted in $r_{opt} = 127.31$, $r_5 = 31.68$, and $r_7 = 42.65$ actions per hour.

Figure 2 summarizes the resulting CE flux mediated by CETP and the esterification of FC by LCAT in the lipoprotein fractions. The model predicts a CE net flux mediated by CETP of 12.8 and 16.5 µM/h out of LDL and HDL and...
TABLE 2. Differences in VLDL, IDL, LDL, and LDL subfraction compositions of plasma stored at 4°C or 37°C for 1 h before lipoprotein isolation (n = 15)

| Fraction | VLDL | IDL | Total LDL | LDL-I | LDL-II | LDL-III |
|----------|------|-----|-----------|-------|--------|---------|
| Density (g/ml) | 0.950–1.006 | 1.006–1.019 | 1.019–1.063 | 1.019–1.034 | 1.034–1.040 | 1.040–1.063 |
| PL per ApoB MD (quartiles) | baseline | 2,583 (2,079, 2,729) | 1,298 (1,212, 1,372) | 900 (860, 981) | 1,055 (976, 1,093) | 904 (861, 960) |
| MD difference (95% CI) | 29.3 (21.7, 21.1) | 11.8 (2.2, 4.4) | 11.0 (4.4, 13.0) | 18.5 (41.0, 29.1) | 22.0 (35.0, 12.8) |
| CE per ApoB MD (quartiles) | baseline | 1,668 (1,342, 1,956) | 979 (875, 1,036) | 807 (688, 831) | 923 (855, 958) | 785 (706, 819) |
| MD difference (95% CI) | 35.2 (25.2, 33.0) | 12.4 (4.4, 9.5) | 25.7 (17.6, 38.2) | 8.5 (17.0, 35.9) | 25.2 (31.4, 19.4) |
| ApoB in mg/dl | baseline | 2,158 (1,592, 2,247) | 2,270 (1,697, 2,498) | 2,152 (1,957, 2,191) | 2,169 (2,105, 2,260) | 1,889 (1,711, 1,955) |
| MD difference (95% CI) | 264 (211, 318) | 9.0 (27.2, 38.2) | 8.8 (18.6, 31.1) | 8.5 (17.0, 35.9) | 22.3 (31.4, 19.4) |

CI, confidence interval; MD, median difference denotes the difference between plasma kept for 1 h at 37°C and baseline. The P value indicates the significance of change (Wilcoxon signed-rank test).

Parameter inference, step 2

The parameters CETP<sub>TG</sub>, rcetp<sub>TG</sub>, r<sub>TG</sub>, and r<sub>5</sub> are adopted from step 1. Optimization yielded r<sub>1</sub> = 2.22, r<sub>2</sub> = 0.0038, and r<sub>6</sub> = 0.0059, and r<sub>7</sub> = 0.0001 per hour.

The lipid load of lipoprotein particles entering the LDL density range (the starting point) amounts to 540 TG, 2,385 CE, and 953 FC molecules per particle, respectively. Based on the parameter λ, the model suggests ApoB production of 1.17 mg/dl per hour in the LDL fraction.

The fitted values are 192, 146, and 154 TG per ApoB; 2,338, 2,128, and 1,800 CE per ApoB; 905, 789, and 616 FC per ApoB; and 23.7, 20.7, and 16.5 mg/dl ApoB in LDL-I, LDL-II, and LDL-III, respectively. This concurs well with the observed values (Table 2).

Lipid fluxes

Figure 3 summarizes the flux of lipids in the LDL fractions predicted by the model. The model suggests that there is a net loss of 3.6 µM/h FC, 5.6 µM/h CE, and 5.7 µM/h TG in LDL, which is not caused by endocytosis. The corresponding loss due to endocytosis in the LDL fraction amounts to 16.8 µM/h FC, 45.5 µM/h CE, and 5.9 µM/h TG, respectively. Selective efflux of CE amounts to 8.4 µM/h.

Table 4 relates fluxes of TG, FC, and CE into and out of lipoprotein fractions estimated by our new model with data from the literature.

DISCUSSION

Aided by measured compositional changes in plasma in vitro, the model presented here is able to calculate rate constants for the LDL steady state in vivo. Relevant enzyme activities and metabolic fluxes of TG, CE, and FC in LDL can be estimated. Further, the flux of TG mediated by CETP and the flux of CE mediated by LCAT and CETP in the VLDL, LDL, IDL, and HDL fraction can be assessed. Here we present for the first time an estimation of the selective CE efflux in LDL.
TABLE 3. Differences in total plasma, LDS, HDL, and HDL subfraction of plasma stored at 4°C or 37°C for 1 h before lipoprotein isolation (n = 15)

| Density (g/ml) | 1.063–1.210 | 1.063–1.100 | 1.100–1.125 | 1.125–1.210 | >1.210 |
|---------------|-------------|-------------|-------------|-------------|--------|
| MD difference | +1.3 (0.9, 1.7) | +5.2 (0.6, 10.7) | -20.6 (15.7, 25.6) | +5.7 (3.3, 8.1) | -3.6 (7.3, 10.0) |
| 95% CI        |             |             |             |             |        |

**FCS in µM**

| Density (g/ml) | 1.063–1.210 | 1.063–1.100 | 1.100–1.125 | 1.125–1.210 | >1.210 |
|---------------|-------------|-------------|-------------|-------------|--------|
| MD difference | +12.0 (10.8, 17.8) | +3.4 (2.9, 5.1) | +5.1 (3.1, 7.0) | +1.7 (0.4, 2.8) | +5.7 (4.2, 11.1) |
| 95% CI        |             |             |             |             |        |

**TGs in µM**

| Density (g/ml) | 1.063–1.210 | 1.063–1.100 | 1.100–1.125 | 1.125–1.210 | >1.210 |
|---------------|-------------|-------------|-------------|-------------|--------|
| MD difference | +36.3 (12.0, 46.0) | +9.1 (4.4, 22.8) | +18.1 (8.4, 30.9) | +1.7 (0.3, 2.8) | +5.7 (4.2, 11.1) |
| 95% CI        |             |             |             |             |        |

**CEs in µM**

| Density (g/ml) | 1.063–1.210 | 1.063–1.100 | 1.100–1.125 | 1.125–1.210 | >1.210 |
|---------------|-------------|-------------|-------------|-------------|--------|
| MD difference | +36.3 (12.0, 46.0) | +9.1 (4.4, 22.8) | +18.1 (8.4, 30.9) | +1.7 (0.3, 2.8) | +5.7 (4.2, 11.1) |
| 95% CI        |             |             |             |             |        |

**FCs in µM**

| Density (g/ml) | 1.063–1.210 | 1.063–1.100 | 1.100–1.125 | 1.125–1.210 | >1.210 |
|---------------|-------------|-------------|-------------|-------------|--------|
| MD difference | +12.0 (10.8, 17.8) | +3.4 (2.9, 5.1) | +5.1 (3.1, 7.0) | +1.7 (0.4, 2.8) | +5.7 (4.2, 11.1) |
| 95% CI        |             |             |             |             |        |

**ApoB in mg/dl**

| Density (g/ml) | 1.063–1.210 | 1.063–1.100 | 1.100–1.125 | 1.125–1.210 | >1.210 |
|---------------|-------------|-------------|-------------|-------------|--------|
| MD difference | +2.5 (0.9, 3.4) | +1.0 (0.3, 1.7) | +1.0 (0.6, 2.3) | +0.3 (0.1, 0.5) | +0.5 (0.1, 1.0) |
| 95% CI        |             |             |             |             |        |

**ApoA1 in mg/dl**

| Density (g/ml) | 1.063–1.210 | 1.063–1.100 | 1.100–1.125 | 1.125–1.210 | >1.210 |
|---------------|-------------|-------------|-------------|-------------|--------|
| MD difference | +2.5 (0.9, 3.4) | +1.0 (0.3, 1.7) | +1.0 (0.6, 2.3) | +0.3 (0.1, 0.5) | +0.5 (0.1, 1.0) |
| 95% CI        |             |             |             |             |        |

In our approach, particles in the LDL range are uniquely described by the number of CE, FC, and TG molecules. Particle size, density, and its amount of PL molecules can be estimated with this quantities similar to Hübner et al. (23).

We describe the impact of in vitro storage at 37°C for 1 h on unmodified fasting plasma with a high resolution referring to the LDL and HDL subfractions. Although the incubation time is short in relation to the LDL’s retention time, there are significant changes in LDL composition, as illustrated in Table 2. We assume that no HL or LPL action occurs in vitro. Our data support this, as there is no significant net loss in TG.

Yen et al. (12) demonstrated that esterification in LDL and HDL during plasma storage at 37°C is linear for at least 1 h. Furthermore, Diedinger and Kostner (34) studied LCAT activity in plasma and found a nearly linear behavior of FC esterification for 1 h storage at 37°C. Data from Liu and Bagdade (35), as well as data derived by our group (not shown), reveal evidence that the net change in LDL-TG due to CETP action during plasma storage at 37°C is linear for at least 4 h. Thus, the change in lipids in LDL after 1 h of storage of plasma at 37°C is assumed to be similar to the corresponding change in vivo mediated by CETP and LCAT.

Selective CE efflux

Our model predicts a selective CE efflux of 8.4 µM/h out of LDL. To our knowledge, there are no other proven estimations of this reaction’s quantity in vivo. This model predicts that efflux is necessary for the loss of CE from LDL during their retention and hence for the decrease in LDL size, as CETP and LCAT action cause a net flux of 2.7 µM/h CE into the LDL fraction. This supports reports claiming that selective CE efflux in LDL is important to the metabolism of plasma cholesterol. The review of Rains and Brissette (36) claims that the majority of LDL CE enters HepG2 cells via selective efflux. Although this pathway might play a key role in LDL-cholesterol metabolism, there is little evidence thereof.

LCAT

It is reported that 50–120 µM FC in vitro is esterified per hour in plasma at 37°C (37). In our experiments, the total loss of FC in the VLDL, IDL, LDL, HDL, and LDS fractions after 1 h storage at 37°C amounts to 44.0 µM. The corresponding loss measured in total plasma amounts to 55.1 µM. Our model suggests that 27% of FC esterified by LCAT in plasma during the 1 h storage at 37°C is caused by LCAT β-activity. According to Yen et al. (12) HDL is the main substrate for LCAT, while LDL plays a minor role. However, Schwartz et al. (19) estimated that 30% of total plasma CE is produced by LCAT β-activity on VLDL, IDL, and LDL, in accordance with our data.

HLS

While the TG content per LDL (as well as the TG/CE ratio) decreases from LDL-I to LDL-II, the TG content in the LDL-III fraction is significantly higher than in the LDL-II fraction. To model this factor, we tested several
This function leads to an inhibition of the HL action in LDL particles proportional to their density. Small LDL particles consequently lose relatively few or no TG and may be enriched in TG by CETP. However, the model still predicts too little TG per ApoB in the LDL-III fraction.

Malmendier and Berman (21) determined the fractional catabolic rate (FCR) of TG and ApoB in LDL in normolipidemic subjects using double tracers. They report an FCR of $\sim 0.18$ pools/h for TG. The model we are presenting predicts a corresponding FCR of 0.13 pools/h, where 70% of TG loss is caused by HL.

Van Schalkwijk et al. (24) and Hübner et al. (23) introduced mathematical models describing the TG metabolism of ApoB-containing particles in plasma. Lipolysis rates for LDL between 0.0013 and 0.0229 pools/h were estimated in the van Schalkwijk model. However, van Schalkwijk did not consider the net flux of TG mediated by CETP into LDL, which is substantial (as illustrated in Table 2). So instead of TG hydrolysis, the net loss of TG was estimated.

Hübner estimated a lipolysis rate of 0.35 pools/day. However, this rate refers not to LDL, but rather to all ApoB-containing particles and may strongly depend on VLDL lipolysis by LPL.

Changing the HL parameter $r_1$, our model suggests a negative correlation between LDL density and the HL reaction rate. This is in accordance with findings by Zambon et al. (38), who demonstrated that LDL buoyancy and size are inversely associated with HL activity levels in normolipidemic subjects.

CETP

Liu and Bagdade (35) measured in vitro CETP exchange rates in healthy subjects with physiological lipoprotein distribution. They estimated that in LDL 21.3 ± 14.3 µM TG is acquired by CETP within 2 h. Hence, our observation that 8.69 ± 6.2 µM TG is acquired in LDL after 1 h storage at 37°C reveals good agreement.

Fig. 2. Flux of CE due to CETP and LCAT between lipoprotein fractions. CE flux mediated by LCAT and CETP estimated by the model in µM/h. CETP loses CE in LDL and HDL and is estimated by the difference in estimated CE change and CE change observed after 1 h of plasma storage at 37°C. LCAT’s contribution to IDL and VLDL is disregarded. For the CETP reaction, CE hetero- (single arrows) and homoexchange (double arrows) are displayed. As the CETP reaction is assumed to be equimolar, the TG heteroexchanges (not shown) correspond to the opposite CE heteroexchanges.

CETP action in our model leads to CE flux out of but not into the LDL fraction. Moreover, due to model construction in the models of Schwartz and Ouguerram, CETP mediates only a transfer of CE between HDL and LDL but not between VLDL and LDL, which is an important sink for LDL CE in our approach. However, as the review by Barter et al. (39) mentions, there are numerous reports describing such a net flux of LDL CE to VLDL.

Fig. 3. Predicted flux of TGs, FC, and esterified cholesterol in the LDL fraction. Flux is represented in µM/h. For the CETP reaction and the FC influx and efflux reaction, only the net flux is displayed. Besides erythrocytes, cellular components also include FC of lipoproteins like nascent VLDL and chylomicrons, which are transferred to LDL via aqueous diffusion.
Guérin et al. (40) determined the flux of labeled CE on HDL to other lipoprotein fractions in normolipidemic subjects, reporting a flux of 86.6 ± 31.3 µM/h CE from HDL to LDL and 31.8 ± 7.2 µM/h CE from HDL to VLDL + IDL. The model we are presenting predicts that 64.81 µM/h and 23.76 µM/h CE mass is transferred from HDL to LDL and VLDL + IDL, respectively. Although this is slightly smaller mass than that reported by Guérin, note that the ratio of both fluxes is similar.

In our model, 14.8% of CETP-mediated transport in the LDL fraction leads to a CE heteroexchange (one CE is exchanged for one CETP bound CE). The CE homoexchange (one CE for one CETP molecule) is the dominant CETP reaction on LDL particles (77.9%). For a CETP molecule, the model predicts a probability of 0.49, 0.37, 0.11, or 0.03 that the next action involves an HDL, a LDL, a VLDL, or an LDL particle, respectively. Thus, although HDL has less mass than LDL in plasma, CETP prefers HDL, as the total surface of HDL particles in plasma is larger than the corresponding LDL surface. HDL accounts for 51% of CE in the CETP pool and is the main source of CE transported via CETP to VLDL.

CETP inhibition via torcetrapib or anacetrapib causes a strong increase in the molar TG/CE ratio of VLDL (41, 42). This is consistent with the molar change in VLDL after storing plasma for 1 h at 37°C. Hence, it is likely that under CETP inhibition, the lipid load of lipoproteins entering the LDL density range as well as the parameter $CETP_{TG}$ differ strongly from the corresponding values in the noninhibited case. Thus, the reduction in the CETP rate in our model is an unsuitable method to simulate the effect of CETP inhibition on LDL.

A postprandial state can be simulated by increasing the parameter $CETP_{TG}$ as intestinally derived chylomicrons are loaded with a lot of TG and are a good CETP substrate, given the assumption that the starting points are only weakly affected by the postprandial state. Increasing $CETP_{TG}$ leads to a higher TG/CE ratio in LDL. This concurs with the report of Callow et al. (43).

Although the actual dynamics of neutral lipid exchange in plasma via CETP are still not fully understood and factors like the lipid transfer inhibitor protein (13) have been neglected, the submodel of CETP presented here demonstrates good agreement with flux data reported in the literature. An important property is that the model grants mass balance of CE and TG between the lipoprotein fractions.

Both experimental data (35) and our model suggest that CETP mediates a net flux of CE to VLDL, which has a higher hepatic uptake rate (44). Furthermore, LCAT activity on LDL is responsible for more than a third of total FC esterification in plasma. Thus, our results suggest that besides HDL, LDL is also important for the flux of cholesterol from the periphery to the liver.

It is beneficial to consider the dynamics of FC, TG, CE, and ApoB in the LDL subfractions instead of focusing on only a part of those species, as they are linked by reactions like LCAT and CETP. This model predicts that selective CE efflux is important for the shrinkage of LDL particles and is able to estimate its quantity in vivo. Furthermore, it supports the hypothesis that LCAT activity on LDL occurs to a significant degree in vivo (19). We are also able to describe the flux of TG and CE mediated by CETP between all lipoprotein species.

The lifetime of LDL particles expressed by the FCR of ApoB is an important measure in the kinetic studies in lipoproteins. However, it was fixed in the model we are describing (parameter $\lambda$). This was done because parameter inference in step 2 gave us a wide range of potential FCRs. However, fixing a parameter in the step 2 of parameter inference such as the HL parameter $\eta$ due to a priori knowledge might enable us to estimate the FCR.

### Influence of measurement noise on parameter estimation

An important issue in model evaluations is the measurement noise, as small changes in lipid composition in vitro at 37°C are used to estimate lipid fluxes and reaction rates. Corruption by noise prevents the use of this model on individuals, but considering the mean of a group of bands, it is possible to minimize measurement noise. Furthermore, we used duplicate samples per person for each time point to address both the variability of sample preparation and imprecision of the measurement itself.

Noise is mainly generated by the gradual isolation of lipoprotein fractions and subsequent measurement procedure. Additional experiments (data not shown) were performed to estimate the coefficient of variation (CV) in plasma,

### TABLE 4. Literature-expected and model-derived lipid fluxes

| Reaction | Model Value | Published Value | Reference |
|---------|-------------|-----------------|-----------|
| β-LCAT (FC in µM/h) | 15.62 | 18.9 | Schwartz et al. (19) |
| Total LCAT (FC in µM/h) | 58.12 | 50–120 | Glomset (37) |
| HL (LDL TG pools/day) | 3.19 | 4.32 | Malmendier and Berman (21) |
| CETP CE out of LDL (µM/h) | 131.2 | 130.0; 111.77 ± 41.07 | Schwartz et al. (19); Ougueram et al. (20) |
| CETP CE into of LDL (µM/h) | 118.4 | 137.5; 110.83 ± 41.62 | Schwartz et al. (19); Ougueram et al. (20) |
| CETP CE from HDL to IDL + VLDL (µM/h) | 23.76 | 31.81 ± 7.2 | Guérin et al. (40) |
| CETP CE from HDL to LDL (µM/h) | 64.81 | 86.6 ± 31.3 | Guérin et al. (40) |
| Selective CE efflux (µM/h) | 8.39 | — | — |

*aThere are no published data on estimated selective CE efflux from LDL.*
The CVs of CE, FC, TG, and PL are <2.5% in plasma in total, LDL, and lipido-ApoB ratio in LDL-I, LDL-II, and LDL-III. In the other fractions, it is <3, 5.5, 4, and 5% for CE, PL, TG, and FC, respectively. The corresponding CV is high especially when the concentration is low. For ApoB, all CVs are lower than 3%. Averaging two measurements, as we did here, decreases the CV by ~30%. In general, using the mean of n samples decreases the CV by the factor of $1/\sqrt{n}$.

The three most important changes observed after 1 h of storage at 37°C are the following: 1) change in TG per ApoB in LDL-I, LDL-II, and LDL-III (to estimate CETP activity); 2) change in CE concentration in the combined VLDL, IDL, LDL, HDL, and LDL fraction (to estimate total LCAT activity); and 3) change in CE concentration in the combined VLDL, IDL, and LDL fraction (to estimate “LCAT on LDL” activity).

Observed changes account for 6.5, 1.6, and 1.2%, respectively. Even using duplicate samples, the corresponding CVs per individual would amount to 1.63, 1.12, and 1.07%, respectively.

However, using n = 15 samples in duplicate from a homogeneous group, the corresponding CVs are reduced to ~0.42, 0.29, and 0.28%, respectively.

The first effect is way beyond the measurement imprecision; the second effect is highly significant ($P = 0.003$, Wilcoxon signed-rank test). Although relatively small, the small change in CE in the combined VLDL, IDL, and LDL fraction remains significant ($P = 0.047$, Wilcoxon signed-rank test). Nevertheless, its 95% confidence interval in our n = 15 group is 6.29–52.01 µM. Hence, the influence of noise on the change in CE in the combined VLDL, IDL, and LDL fraction is clearly lower in the validation group than in the model group due to an exceptional outlier. Consequently, the rates and fluxes dependent on these changes in CE are much lower than in the model group, namely LCAT activity in plasma and LDL, CE efflux, and FC efflux. CETP flux data, TG hydrolysis, and the starting points and the mass of lipids entering and leaving the LDL fraction are all very similar.

**Estimation of variability of reaction rates and lipid fluxes**

To make a rough estimate of the variability of the reaction rates and lipid fluxes, we calculated the individual reaction rates in each of the 15 volunteers. As discussed before, due to the overlap of measurement noise and the real effect in certain lipid changes, individual estimations may be seriously corrupted by measurement imprecision, and the variation in calculated reaction rates should reflect the model’s variability.

To reduce the influence of measurement imprecision on this estimation, we adjusted the data after a 1 h storage at 37°C in the following way. Being aware of the measurement noise, we could estimate its contribution to the SD in the change in lipids. We reduced the corresponding SD of change observed by the corresponding proportion, while keeping the mean. As this method and the adjustment are relatively vague and few cases are considered, this estimation of the variability of reaction rates is rather imprecise and may only produce an upper limit for the genuine variability in reaction rates and fluxes.

Table 5 shows this estimation’s result. While CETP, FC efflux, combined FC efflux (reaction 5 + 7), and HL reactions reveal a relative small range of variability, reactions highly affected by measurement noise such as selective CE efflux and β-LCAT exhibit greater variability. These CE-related reactions have a relatively weak metabolic rate in relation to the bulk of CE in an LDL particle and are barely mentioned in the literature.

Comparing individual reaction rates between the 16 males and the 9 women in the model and the validation group, the only gender-specific difference we observed was in HL ($P = 0.002$, Mann-Whitney U test). This amounted to $r_1 = 2.57$ and $r_1 = 1.12$ for men and women, respectively. This difference concurs closely with the literature (45). Here the HL activity of white women is on average 56% lower than that in white men.

**Summary/outlook**

In summary, we have provided a detailed description of the LDL metabolism in normolipidemic humans. Our results are well in line with previously reported results, which are derived with more elaborate methods (i.e., the use of
The predicted rates and fluxes are derived by the averaged profile (n = 15). To estimate uncertainty, we calculated rates and fluxes of each individual using the corresponding baseline lipid profile and an adjusted (in respect to the measurement imprecision) lipid profile after 1 h storage at 37°C.

TABLE 5. Estimation of uncertainty of rates, parameters, and model-derived fluxes (n = 15)

| Fluxes and Rates Derived by the Averaged Lipid Profile | Median (Quartiles) of Individually Calculated Fluxes and Rates |
|--------------------------------------------------------|---------------------------------------------------------------|
| Measured esterification (µM/h)                          |                                                               |
| Total esterification in plasma                          |                                                               |
| Reaction rates and the parameter CETP<sub>TG</sub>       |                                                               |
| r<sub>1</sub>                                            | 2.22                                                          | 2.07 (1.42, 3.35)                                              |
| r<sub>2</sub>                                            | 0.0038                                                        | 0.0018 (0.0001, 0.0087)                                        |
| r<sub>3</sub>                                            | 20.7                                                          | 19.2 (16.0, 25.1)                                              |
| r<sub>4</sub>                                            | 107                                                           | 100 (80.6, 127)                                               |
| r<sub>5</sub>                                            | 31.7                                                          | 31.0 (0, 64.8)                                                |
| r<sub>6</sub>                                            | 29.5                                                          | 35.1 (30.2, 38.1)                                              |
| r<sub>7</sub>                                            | 42.6                                                          | 55.7 (9.37, 75.5)                                              |
| r<sub>8</sub>                                            | 74.3                                                          | 82.6 (77.0, 87.5)                                             |
| r<sub>8</sub>                                            | 127                                                           | 124 (100, 148)                                                |
| CETP<sub>TG</sub>                                       | 0.165                                                         | 0.151 (0.115, 0.198)                                           |
| CETP-mediated TG net flux (µM/h) (compare with Fig. 1)   |                                                               |
| To LDL                                                  | 11.2                                                          | 11.8 (6.62, 14.9)                                              |
| To HDL                                                  | 14.7                                                          | 13.5 (11.7, 17.4)                                              |
| To VLDL                                                 | −23.7                                                         | −19.9 (−32.7, −15.3)                                           |
| To IDL                                                  | −2.00                                                         | −2.57 (−3.11, −1.06)                                           |
| Initial composition LDL (molecules per LDL particle)     |                                                               |
| TG                                                      | 540                                                           | 622 (364, 829)                                                |
| CE                                                      | 2.385                                                         | 2.531 (2.368, 2.653)                                           |
| FC                                                      | 952                                                           | 906 (766, 933)                                                |
| Lipid fluxes in and out of LDL (compare with Fig. 2) (µM/h) |                                                               |
| TG influx by LDL production                             | 11.6                                                          | 12.4 (6.63, 16.9)                                              |
| CE influx by LDL production                             | 51.1                                                          | 50.2 (38.1, 59.9)                                              |
| FC influx by LDL production                             | 20.4                                                          | 16.7 (14.4, 20.6)                                              |
| TG efflux by endocytosis                                | 5.89                                                          | 7.63 (3.71, 8.82)                                              |
| CE efflux by endocytosis                                | 45.5                                                          | 43.2 (39.9, 49.7)                                              |
| FC efflux by endocytosis                                | 16.8                                                          | 16.1 (13.7, 18.6)                                              |
| FC influx                                               | 33.0                                                          | 34.7 (30.7, 43.5)                                              |
| FC efflux (CE influx) by LCAT                            | 15.6                                                          | 16.5 (0, 28.9)                                                |
| FC efflux                                               | 21.0                                                          | 25.3 (4.41, 34.2)                                              |
| FC efflux + FC efflux by LCAT                            | 36.6                                                          | 36.1 (33.6, 43.1)                                              |
| TG net flux by CETP                                     | 12.8                                                          | 11.8 (6.62, 14.9)                                              |
| Selective CE efflux                                     | 8.39                                                          | 7.49 (0.15, 22.0)                                              |
| Hydrolysis of TG                                        | 18.5                                                          | 16.7 (9.52, 22.4)                                              |

The predicted rates and fluxes are derived by the averaged profile (n = 15). To estimate uncertainty, we calculated rates and fluxes of each individual using the corresponding baseline lipid profile and an adjusted (in respect to the measurement imprecision) lipid profile after 1 h storage at 37°C.

It is possible to gather valuable information and to compile reasonable lipid flux estimations based on an accurate description of the mechanisms leading to changes in LDL particles implemented in our combined in vitro and in silico model. This information is derived from a single blood sample instead of elaborate tracer kinetics with multiple time points and blood draws.

However, the method presented here relies on great precision when measuring lipids and apolipoproteins, as it is supported by small compositional changes in lipoproteins during a 1 h reaction time at 37°C. To ensure the necessary precision, it is necessary to average several measurements. In contrast to the tracer kinetics approach, this method is not suitable for application in individuals but rather for addressing population-based questions.

Kinetic data of LDL lipids and CETP transport can be estimated with relatively moderate effort. Moreover, our model may prove to be a useful tool for investigating the postprandial state, which may not be easily accessible by tracer kinetics.

This model may also be applied to obtain deeper insights into the pathologies of major diseases associated with a perturbed lipoprotein profile like diabetes and CVDs. Furthermore, a refined version of this model might become a helpful tool for diagnostic purposes such as individual risk analysis once measurement uncertainties are minimized.

The inclusion of VLDL and IDL is beneficial. However, they were omitted in our model, as the features of VLDL and IDL differ strongly from LDL. For example, various apolipoproteins besides ApoB influence their metabolism, and the retention time of VLDL and IDL is much shorter than that of LDL.

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