**Supplemental Materials and Methods**

***GC/MS measurements of mass isotopomer distribution*** **-** Cholesterol trimethylsilylether derivatives were separated with a Trace MS plus GC/MS (Interscience, Breda, the Netherlands), using a 20 m × 0.18 mm (0.18 μm film thickness) DB17 ms column (J&W Scientific, Falson, CA). The oven temperature was programmed from 140 to 280°C at 20°/min. A splitless injection was applied. Ions monitored were m/z 458-465 corresponding to the $m_0$-$m_8$ mass isotopomers. The fractional isotopomer distribution measured ($m_0$-$m_8$) was corrected for the fractional distribution due to natural abundance of $^{13}C$ and $^{2}H$ by multiple linear regression as described by Lee *et al.* (1) to obtain excess fractional distribution of mass isotopomers ($M_0$-$M_8$) resulting from isotope dilution and isotope incorporation of infused labeled compounds. In this approach, $M_5$ represented the fractional contribution of the orally administered label ($f^{or}$) and $M_7$ the fractional contribution of the IV administered label ($f^{iv}$), whereas $M_1$ and $M_3$ were used in MIDA calculations of the contributions of newly synthesized cholesterol ($f^{new}$).

***Calculations of kinetic parameters*** **-** Empirically it was found that the decay curves of the fractional contribution of IV-administered cholesterol-$D_7$ in blood could be described appropriately by a two-function equation, *i.e.* :

$$f(t) = f_1^{iv} e^{-k_1 t} + f_2^{iv} e^{-k_2 t} \qquad \text{eq. 1}$$

in which *f* is the fractional contribution of cholesterol-$D_7$ (%) in blood cholesterol, *k* is the rate constant ($h^{-1}$), and *t* represents the time (h). The two subscripts 1 and 2 refer to the two independent sub-functions. For each individual mouse, an optimal fit for the

decay curve was generated using SAAM II software (SAAM Institute, Inc., Seattle, WA, USA), using the time points of t=9 hours and onwards. By extrapolation, the theoretical fractional enrichment at time point zero (*i.e.* the initial concentration *f(0)*) was estimated. This extrapolation assumes that all labels were introduced in the sampled pool at once and were diluted instantaneously:

$$f^{iv}(0) = f_1^{iv} + f_2^{iv} \qquad \text{eq. 2}$$

Using the parameters described above, a number of other kinetic parameters could be calculated. The concentration of IV-administered cholesterol at time point zero ($C_0^{IV}$) could be calculated by:

$$C_0^{iv} = C^{tot} \times f^{iv}(0) \qquad \text{eq. 3}$$

in which $C^{tot}$ represents plasma cholesterol concentration (µmol $l^{-1}$). The ratio of the IV-administered dose of cholesterol-$D_7$, $D^{iv}$ (µmol $kg^{-1}$), and the initial concentration gives the apparent volume of distribution of cholesterol (V (l $kg^{-1}$)):

$$V = \frac{D^{iv}}{C_0^{iv}} \qquad \text{eq. 4}$$

The rapidly exchangeable free cholesterol pool size, $A^{tot}$ (µmol $kg^{-1}$), is calculated as the product of plasma cholesterol concentration and apparent volume of distribution:

$$A^{tot} = C^{tot} \times V \qquad \text{eq. 5}$$

The total turnover rate, $R$ (µmol $kg^{-1}$ $min^{-1}$) consists of two separate turnover rates *i.e.*:

$$R = R_1 + R_2 \qquad \text{eq. 6}$$

from which each of these two parameters (x equals 1 or 2) is represented by:

$$R_x = k_x \times A^{tot} \quad \text{eq. 7}$$

Equations 6 and 7 give:

$$R = A^{tot} \times \left(k_1 + k_2\right) \quad \text{eq. 8}$$

Metabolic clearance rate, *MCR* (ml $kg^{-1}$ $min^{-1}$), could be calculated as the ratio of the total turnover rate and plasma cholesterol concentration:

$$MCR = \frac{R^{tot}}{C^{tot}} \quad \text{eq. 9}$$

Finally half-life time, $t_{½}$ (min) was calculated as:

$$t_{½} = \frac{\ln(2)}{k_x} \quad \text{eq. 10}$$

***Fractional cholesterol absorption measurement*** **-** Cholesterol absorption was measured using the plasma dual isotope ratio method (2). Blood spots obtained at 72 hours after intravenous and oral administration were used for the calculation of fractional cholesterol absorption (3). Fractional cholesterol absorption ($F_a$) was calculated as the ratio of the fraction orally-administered cholesterol-$D_5$ ($f^{or}$) and the fraction IV-administered cholesterol-$D_7$ ($f^{iv}$) as measured in bloodspots obtained 72 hours after adminstration, after being corrected for its orally and IV administered dose, ($D^{or}$) and ($D^{iv}$), respectively:

$$F_a = \frac{f_{72}^{or} / D^{or}}{f_{72}^{iv} / D^{iv}} \times 100\% \quad \text{eq.11}$$

***Mass isotopomer distribution analysis (MIDA) -*** To determine *de novo* cholesterol synthesis, the MIDA approach was used. The theoretical background of this technique is described in detail elsewhere (4-6). In short, the MIDA algorithm allows us to estimate the fractional enrichment of newly synthesized cholesterol in a not accessible pool, *i.e.* in the liver. The first step of this algorithm is the calculation of the ratio of relevant mass-isotopomers distributions (*i.e.*, $M_1$ and $M_3$) due to incorporation of the precursor [$^{13}$C]-acetate. By comparing these ratios to theoretical multinomial curves, in which precursor pool enrichment is plotted against the ratios of $M_1$ and $M_3$, the corresponding precursor pool enrichment can be estimated. Next, from a curve in which $M_1$ and $M_3$ are plotted against precursor pool enrichment, mass-isotopomer distributions of the newly synthesized cholesterol can be estimated ($M_1^{new}$ and $M_3^{new}$). The ratio of the measured fractional distribution of cholesterol and the estimated fractional distribution in the newly synthesized cholesterol gives the fractional contribution of newly synthesized in the cholesterol pool ($f^{new}$):

$$\left(f^{new}\right)_t = \frac{\left(M_1\right)_t}{\left(M_1^{new}\right)_t} = \frac{\left(M_3\right)_t}{\left(M_3^{new}\right)_t} \qquad \text{eq.12}$$

Using multiple sampling in time combined with SAAM II software (SAAM Institute, Inc., Seattle, WA, USA), the synthesis rate constant ($k^{new}$) and the fractional contribution of cholesterol at infinite time ($f^{new}_{\infty}$) can be calculated from the resulting curve. For the calculation of absolute cholesterol synthesis rates, the following non-steady-state equation is used:

$$R^{new} = \frac{k^{new} \times f_t^{new} \times A^{tot}}{\left(1 - e^{-kt}\right)}$$

eq.13

and at infinite time:

$$R^{new} = k^{new} \times f_{\infty}^{new} \times A^{tot} \qquad \text{eq.14}$$

***Determination of the sources of fecal neutral sterols –*** To calculate fecal excretion of blood-derived cholesterol, we adapted the method described for use in humans by Ferezou *et al.* (7). This method enables us to calculate the percentage of fecal neutral sterol excretion that is derived from the blood compartment. For this calculation, we measured the mean enrichment of fecal cholesterol-$D_7$ in cholesterol extracted from feces from day 2-5 of the experiment. This value was divided by the enrichment of cholesterol-$D_7$ in blood spots 24 h before the midpoint of the feces collection (i.e. day 2), in order to take into account the intestinal transit. The equation used for calculation of this fractional fecal excretion of blood-derived cholesterol ($^{blood}\varepsilon$) is as follows:

$$^{blood}\varepsilon = \frac{\overline{^{feces}f^{iv}_{24-120}}}{^{spot}f^{iv}_{48}} \qquad \text{eq.15}$$

The absolute cholesterol loss from blood ($^{blood}$E) is the product of eq.15 and the total fecal loss of neutral sterols ($^{total}$E):

$$^{blood}\mathrm{E} = {}^{blood}\varepsilon \times {}^{total}\mathrm{E} \qquad \text{eq.16}$$

To determine the fraction of biliary cholesterol that is derived from the blood compartment, we measured the enrichment of intravenously administered cholesterol-$D_7$ in blood spots and bile samples at 24 h after administration. The fraction of biliary cholesterol that is derived from the blood compartment ( ${}^{blood}_{bile}\beta$ ) is calculated as:

$$ {}^{blood}_{bile}\beta = \frac{{}^{bile}f^{iv}_{24}}{{}^{spot}f^{iv}_{24}} \qquad \text{eq.17} $$

The remaining fraction of biliary cholesterol consists of cholesterol excreted from the liver without having entered the circulation ( ${}^{liver}_{bile}\beta$ ) and is assumed to be a newly synthesized fraction.

$$ {}^{liver}_{bile}\beta = 1 - {}^{blood}_{bile}\beta \qquad \text{eq.18} $$

Daily biliary cholesterol output during 24h ( ${}^{total}$B), representing the amount of cholesterol that enters the intestinal lumen *via* the bile, was calculated as the product of bile flow (b; µl $kg^{-1}$ $day^{-1}$) and the concentration of cholesterol [chol], as measured during the first hour of bile collection:

$$ {}^{total}\mathrm{B} = b \times [chol] \qquad \text{eq.19} $$

The absolute amount of blood-derived cholesterol that is excreted into the intestinal lumen *via* the biliary pathway is calculated as the product of eq. 17 and 19 and needs to be corrected for fractional intestinal cholesterol reabsorption in order to caculate the amount of blood-derived cholesterol that is excreted into the feces via the biliary pathway ( ${}^{blood}_{bile}\mathrm{E}$ ):

$$ {}^{blood}_{bile}\mathrm{E} = {}^{blood}_{bile}\beta \times {}^{total}\mathrm{B} \times (1 - F_a) $$

eq.20

The difference between the total blood-derived cholesterol in the feces (eq.16) and the fecal blood-derived cholesterol secreted via bile (eq.20) gives the amount of cholesterol in the feces that is excreted from the blood compartment directly into the intestinal lumen ( ${}^{blood}_{intestine}\mathrm{E}$ ):

$$ {}^{blood}_{intestine}\mathrm{E} = {}^{blood}_{total}\mathrm{E} - {}^{blood}_{bile}\mathrm{E} \qquad \text{eq.21} $$

From the product of the fractional contribution of fecal cholesterol that is derived from the blood compartment (eq.15) and the fractional contribution of newly synthesized cholesterol in the blood compartment at the same sampling point, divided by the f-value of newly synthesized cholesterol in feces of day 2-5, the fraction of newly synthesized fecal cholesterol that is derived from the circulation can be calculated

$$ {}^{blood}\varepsilon^{new} = \frac{{}^{blood}\varepsilon \times \left(f^{new}\right)_{48}}{\overline{{}^{feces}f^{new}_{24-120}}} \qquad \text{eq.22} $$

The remaining fraction of newly synthesized cholesterol in feces is not derived from the blood-compartment and can be calculated as follows:

$$ {}^{non-blood}\varepsilon^{new} = 1 - {}^{blood}\varepsilon^{new} \qquad \text{eq.23} $$

The mass of the fraction newly synthesized cholesterol in feces that is derived from the blood-compartment is calculated by multiplying total fecal excretion of blood-derived cholesterol with the f-value in blood spots at infinite time derived from the MIDA calculations (eq. 12):

$$ {}^{blood}\mathrm{E}^{new} = {}^{blood}_{total}\mathrm{E} \times \left( f^{new} \right)_{\infty} \qquad \text{eq.24} $$

Hence, the mass of newly synthesized cholesterol in the feces that is not derived from the circulation is calculated as:

$$ {}^{non-blood}\mathrm{E}^{new} = {}^{blood}\mathrm{E}^{new} \times \left( \frac{{}^{non-blood}_{feces}\varepsilon^{new}}{{}^{blood}_{feces}\varepsilon^{new}} \right) \qquad \text{eq.25} $$

This fraction, synthesized in either enterocytes or hepatocytes, is excreted into the lumen directly *via* the intestine or *via* the bile, respectively. The latter is known as the biliary fraction that is not derived from the circulation. The difference in mass of newly synthesized cholesterol in the feces that is not derived from the circulation and the mass of newly synthesized cholesterol in the feces secreted into the bile directly by the liver, gives the mass of cholesterol synthesized in the enterocytes:

$$ {}^{intestine}\mathrm{E}^{new} = {}^{non-blood}\mathrm{E}^{new} - {}^{liver}_{bile}\mathrm{E} \qquad \text{eq.26} $$

Based on the dietary intake of cholesterol and values of fractional cholesterol absorption, the mass of cholesterol that is not absorbed and eventually ends up in the feces can be calculated:

$$^{non-abs}\mathrm{E} = Intake \times \left(1 - F_a\right) \qquad \text{eq.27}$$

Finally, the fraction of total fecal neutral sterol content that is not excreted via one of the above mentioned pathways, must be derived from shedding of enterocytes or other sources:

$$^{shedding-ao}\mathrm{E} = {}^{total}\mathrm{E} - \left[{}^{blood}\mathrm{E} + {}^{non-blood}\mathrm{E}^{new} + {}^{non-abs}\mathrm{E}\right] \qquad \text{eq.28}$$

**REFERENCES**


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Supplemental Table 1. Primers and probes used for real-time PCR analysis.

| Gene | | Sequence | GenBank™ |
|---|---|---|---|
| *β-actin* | *Forward* | AGC CAT GTA CGT AGC CAT CCA | NM_007393 |
| | *Reverse* | TCT CCG GAG TCC ATC ACA ATG | |
| | *Probe* | TGT CCC TGT ATG CCT CTG GTC GTA CCA C | |
| *Abca1* | *Forward* | CCC AGA GCA AAA AGC GAC TC | NM_013454 |
| | *Reverse* | GGT CAT CAT CAC TTT GGT CCT TG | |
| | *Probe* | AGA CTA CTC TGT CTC TCA GAC AAC ACT TGA CCA AG | |
| *Abcg5* | *Forward* | TCA GGA CCC CAA GGT CAT GAT | NM_031884 |
| | *Reverse* | AGG CTG GTG GAT GGT GAC AAT | |
| | *Probe* | CCA CAG GAC TGG ACT GCA TGA CTG CA | |
| *Abcg8* | *Forward* | GAC AGC TTC ACA GCC CAC AA | NM_026180 |
| | *Reverse* | GCC TGA AGA TGT CAG AGC GA | |
| | *Probe* | CTG GTG CTC ATC TCC CTC CAC CAG | |
| *Npc1* | *Forward* | GGG TGG ACA ACA TCT TCA TTC TAG T | NM_008720 |
| | *Reverse* | CCT GCC CAG CTG CTG ATC | |
| | *Probe* | TCC TCC TGA AGA CGC TCA TCT CTC TGG TA | |
| *Npc1l1* | *Forward* | GAG AGC CAA AGA TGC TAC TAT CTT CA | NM_207242 |
| | *Reverse* | CCC GGG AAG TTG GTC ATG | |
| | *Probe* | ACT CCA GCA AAC ACC GCA CTG CC | |
| *Rab8a* | *Forward* | GCC AAC ATC AAT GTG GAG AAT G | NM_023126 |
| | *Reverse* | GGG CTG TTC CCT TCC AAT TT | |
| | *Probe* | TTC ACT CTT GCC AGG GAT ATC AAA GCA A | |
| *Rab9* | *Forward* | GTT GCA CCC AGG CGT GTT | NM_019773 |
| | *Reverse* | ATA ATG TAA GTT TTG ATT TCG AAT CCT TAG | |
| | *Probe* | CCG CCC CCA GGA CGT CCA | |