In vivo tissue cholesterol efflux is reduced in carriers of a mutation in APOA1®

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Abstract

Atheroprotection by high density lipoprotein (HDL) is considered to be mediated through reverse cholesterol transport (RCT) from peripheral tissues. We investigated in vivo cholesterol fluxes through the RCT pathway in patients with low plasma high density lipoprotein cholesterol (HDL-c) due to mutations in APOA1. Seven carriers of the L202P mutation in APOA1 (mean HDL-c: 20 ± 19 mg/dl) and seven unaffected controls (mean HDL-c: 54 ± 11 mg/dl; P < 0.0001) received a 20 h infusion of 13C2-cholesterol (13C-C). Enrichment of plasma and erythrocyte free cholesterol and plasma cholesterol esters was measured. With a three-compartment SAAM-II model, tissue cholesterol efflux (TCE) was calculated. TCE was reduced by 19% in carriers (4.6 ± 0.8 mg/kg/h versus 5.7 ± 0.7 mg/kg/h in controls, P = 0.02). Fecal 13C recovery and sterol excretion 7 days postinfusion did not differ significantly between carriers and controls: 21.3 ± 20% versus 13.3 ± 6.3% (P = 0.33), and 2.015 ± 1,431 mg/day versus 1456 ± 404 mg/day (P = 0.43), respectively. TCE is reduced in carriers of mutations in APOA1, suggesting that HDL contributes to efflux of tissue cholesterol in humans. The residual TCE and unaffected fecal sterol excretion in our severely affected carriers suggest, however, that non-HDL pathways contribute to RCT significantly.—Holleboom, A. G., L. Jakulj, R. Franssen, J. Decaris, M. Vergeer, J. Koetsveld, J. Luchoomun, A. Glass, M. K. Hellerstein, J. P. Kastelein, G. K. Hovingh, J. A. Kuivenhoven, A. K. Groen, S. M. Turner, and E. S. G. Stroes.

In vivo tissue cholesterol efflux is reduced in carriers of a mutation in APOA1. J. Lipid Res. 2013. 54: 1964–1971.

Epidemiological studies have demonstrated a strong inverse relationship between plasma high density lipoprotein cholesterol (HDL-c) concentrations and the risk of cardiovascular disease (1–4). However, the anti-atherogenic properties of HDL could not be substantiated in a recent meta-regression analysis: pharmacological increases in HDL-c did not translate into a decreased cardiovascular disease risk (5). These findings have emphasized the need for other measures than plasma HDL-c concentrations to assess the atheroprotective properties of HDL. Ideally, such measures should relate directly to the mechanistic pathways that form the basis of the proposed anti-atherogenic effects of HDL in humans (3, 6).

The most frequently studied function of HDL is its role in the reverse transport of cholesterol from peripheral tissues (RCT). RCT has been proposed as the uptake of cholesterol from peripheral cells by nascent HDL particles mainly consisting of lipid-poor apolipoprotein A1 (apoA1), mediated by lipid transporter molecules such as ATP-binding cassette transporter A1 and G1 (ABCA1 and ABCG1) and scavenger receptor type B1 (SR-BI) and the subsequent...
delivery of cholesterol to the liver for excretion into the feces as neutral sterols (NSs) or bile acids (BAs) \( (7) \). Indirect proof for a role of HDL in tissue RCT in humans has come from patients with HDL deficiency syndromes, in whom accumulations of cholesterol in peripheral tissues such as corneas (8), tonsils (9), and glomeruli (8) are a central feature. The contribution of individual components in RCT has been addressed predominantly by in vitro quantification of the cholesterol efflux capacity of macrophages and the capacity of plasma to induce cellular cholesterol efflux, as well as by human studies of cholesterol excretion as BAs and fecal sterols [for review, see (6, 7)]. However, very few studies have addressed in vivo measurements of the RCT pathway in humans. Such studies are highly relevant, considering the current conflicting results regarding the role of HDL in RCT in murine studies \( (10–14) \), as well as in human studies of fecal sterol excretion \( (FSE) \) \( (15–17) \). Major hurdles to resolve this ongoing debate pertain to the complex nature of cholesterol metabolism and accordingly to the methodological complexity of quantifying in vivo cholesterol fluxes in humans.

In the present study, in vivo tissue cholesterol efflux \( (TCE) \) was quantified in carriers of a mutation in \( APOA1 \) as compared with healthy controls. We used a recently developed stable isotope infusion method combined with a three-compartment SAAM-II model \( (18) \). To assess the entire RCT pathway, fecal recovery of the cholesterol tracer and total FSE were measured as well. We demonstrate that TCE is significantly reduced in carriers of mutations in \( APOA1 \) as compared with controls, suggesting that apoA-I and HDL indeed contribute to TCE in humans.

METHODS

Subjects

Subjects were considered a case if they were between 18 and 70 years of age and if they were heterozygous for the functional p.L202P mutation in \( APOA1 \) \( (c.C643T) \) \( (19) \) in our lipid clinic. Control subjects were unaffected and unrelated normolipidemic individuals recruited via advertisements. Subjects were excluded from participation if they had any of the following conditions: a body mass index \( (BMI) \) >35 kg/m\(^2\); alcohol or drug abuse; uncontrolled hypertension defined as systolic pressure >160 mmHg and/or diastolic pressure >100 mmHg; diabetes mellitus; or cardiovascular disease within 6 months prior to inclusion. Lipid lowering drugs (statins, fibrates, nicotinic acid derivatives, ezetimibe) had to be discontinued at least 6 weeks before the screening visit. The study protocol was approved by the Institutional Review Board of the Academic Medical Center, University of Amsterdam, The Netherlands. Each participant provided written informed consent.

Experimental procedures

To ensure stable cholesterol intake, participants maintained a standardized diet from 2 days prior to the infusion until the end of study. Daily cholesterol intake was monitored using a dietary record. In order to normalize the fecal isotope recovery measurements for variations in fecal flow, participants used capsules containing 3 mg \( [2H4] \) stanol (Medical Isotopes Inc., Pelham, AL) three times daily with their meals from 2 days prior until 7 days following infusion. One day postinfusion, participants started collecting daily stool samples for 7 days, using a FSC specimen collection system (Fisher Scientific, Hampton, NH).

On the day of infusion, participants were admitted to the hospital at noon after a light breakfast in the morning. Two intravenous catheters were placed, one used for blood sampling and the other for a 20 h constant infusion of \( ^{13} \)C cholesterol \( (13-C) \). Infusates were prepared by dissolving 200 mg of \( [2,3,15] \) C cholesterol \( (99\%), \) Isotec, Miamisburg, OH) into 13 ml of warm USP ethanol. This solution was mixed slowly into 120 ml of 10% Liposyn III (Hospira Inc., Lake Forest, IL) to a final concentration of 1.5 mg/ml \( ^{15} \) C. The infusate, piggybacked into normal saline (100 ml/h), was started at 3:00 PM and administered over the subsequent 20 h at a rate of 5.5 ml/h. Blood samples were collected directly before the start of infusion and at subsequent hourly intervals. Immediately after drawing, blood was placed on ice. After centrifugation, plasma was aliquoted and stored at -80°C. Every 3 h residual red blood cells (RBCs) were washed with saline twice and stored at -80°C. Two and seventeen hours after start of the infusion, two standardized light meals were served. For clinical applicability, the infusion protocol originally developed by Turner et al. \( (18) \) was slightly modified regarding the duration of \( ^{13} \) C infusion. Turner et al. \( (18) \) examined the effect of infusion time on the kinetics: small but systematic increases in fluxes 1 and 3 were observed as the infusion time was shortened. Although this may influence absolute values, it will not influence the comparison between fluxes in groups receiving the same infusion rates and times. In order to restrict the burden of study participation for our volunteers, we therefore used a 20 h infusion period.

Analytical procedures

Plasma analyses. Total and free cholesterol \( (FC) \), HDL-c, and triglycerides were determined with commercially available enzymatic methods (Wako Diagnostics, Richmond, VA and Roche Diagnostics GmbH, Mannheim, Germany). Low density lipoprotein cholesterol \( (LDL-c) \) was calculated using the Friedewald formula. ApoA-I and apoB were determined by nephelometric immunochemistry (Behring GmbH, Marburg, Germany).

Analysis of cholesterol and its metabolites. Plasma FC was extracted with ethanol-acetone, acetylated with toluene/pyridine/ acetyl chloride, and dissolved in toluene for analysis of isotopic enrichment of \( ^{13} \) C by mass spectrometry \( (MS) \) \( (20) \). FC from RBCs was analyzed after homogenization with silicate beads and extraction in chloroform-methanol. For measurement of cholesterol ester \( (CE) \) enrichment by MS, extracted plasma FC and CE were first separated on an amino-propyl solid phase extraction \( (SPE) \) cartridge, the fatty acid moiety of the CE cleaved by methanolic HCl, and the resultant FC was subsequently acetylated.

Stool samples were first homogenized with an equal mass of water after which NSs and BAs were extracted separately under basic and acidic conditions, respectively, in the presence of the internal standards 5α-cholestone and 5α-cholanic acids. The BA extract was split into two portions. The first, used for compositional analysis by flame ionization detector \( (FID) \), was directly subjected to a two-step derivatization: butylation with butanolic HCl followed by silylation by BSTFA-pyridine. The second portion was further purified for mass spectrometric analysis with an octadecyl SPE cartridge, selectively eluting primarily deoxycholic acid with a 20% aqueous-methanol solution prior to butylation and silylation. The NS fraction was silylated directly for both compositional and isotopic analysis.

Isotopic enrichments of \( ^{13} \) C were measured using gas chromatography \( (GC) \) combustion isotope ratio mass spectrometry (Thermo Finnegan MAT 253 IRMS, Bremen, Germany) and determined as atom percent excess \( (APE) \) by comparison of the in vivo cholesterol efflux in HDL deficiency 1965
unknown samples to a standard curve, generated with gravimetrically prepared working lab standards with known enrichments. Molar percent excess was calculated as 14.5 or $15 \times APE$ for the acetol or silyl derivative of cholesterol, respectively, and by $17 \times APE$ for the butyl-silyl derivative of deoxycholic acid.

Compositional analysis and excretion measurement of BAs and NSs was performed by GC/FID by comparison with the internal standards and sitostanol. GC peak areas of cholesterol, coprostanol, epicoprostanol, coprostan-3-one, and cholestanol were used to calculate NS mass. GC peak areas of isolithocholic, prostanol, epicoprostanol, coprostan-3-one, and cholestanol were used to calculate acidic sterol mass.

**Calculation of cholesterol fluxes.** TCE and additional plasma cholesterol fluxes were calculated by use of a three-compartmental kinetic model (SAAM-II software, University of Washington, Seattle, WA, version 1.2.1). This model’s compartments, assumptions, and equations are summarized in Fig. 1A. Its biological background, development, and validation have been described in detail (18). Briefly, following the description of three compartment models of whole-body cholesterol metabolism (21–23), several groups measured plasma cholesterol dynamics in humans through analysis of multi-compartmental decay curves of radio-isotopically labeled cholesterol (24–27). This established several points. First, rapid equilibration of FC within the plasma lipoprotein compartment as well as with hepatobiliary FC pools occurs within hours. Second, entrance of the vast majority of cholesterol from tissues into blood is in the form of FC. Third, almost all FC enters the plasma compartment on HDL particles. These findings imply that application of a labeled FC constant infusion approach can capture the FC flux rate between unlabeled pools in tissues and the rapidly labeled FC pool, which includes plasma and can therefore be sampled easily.

In this context, the movement of unlabeled FC from slow turnover pools in extrahepatic tissues dilutes the labeled pool in plasma and liver, while labeled plasma FC leaves the circulatory pool for these tissues. These processes represent net FC efflux from extrahepatic tissues into the plasma compartment (TCE) and FC influx into tissues, respectively. Plasma FC can also be esterified by LCAT to CE, which may then exit the circulation. At metabolic steady state, TCE must equal (i.e., be balanced by) FC influx into tissues plus FC esterification.

Our model is based upon several points: 1) subjects are at metabolic steady state (i.e., constant weight and total cholesterol concentrations); 2) each pool is at metabolic steady state (i.e., flux in = flux out); 3) esterification of plasma FC is irreversible; and 4) there is no direct removal of RBC FC.

For simplicity, we have ignored the flux of cholesterol from the CE compartment to the plasma FC compartment via liver and via tissues, because Schwartz et al. (25) reported that these are indeed very small over the time course: the total CE flux to liver is ca. 3 umol/min/70 kg, whereas the FC flux is 45 umol/min/70 kg. Thus, even if all CEs that went to the liver returned as FC, it would only account for 10% of the FC flux. Toward tissues, total CE flux is 0.39 umol/min/70 kg whereas FC flux is 6.8 umol/min/70 kg; theoretical maximal contribution to FC flux is 6%.

Inputs into the model are: 1) the RBC pool size: $V_2 = mg \text{FC/g RBC} \times \text{hematocrit} \times \text{blood volume estimated as 7% of body weight}$; 2) the CE pool size: $V_3 = mg \text{CE/kg plasma volume estimated as 7% of body weight, adjusted for hematocrit}$; 3) the infusion rate of $^{13}$C-CE (B); and 4) the $^{13}$C-CE enrichments of FC sampled in V1, the pool size of plasma FC and its rapidly equilibrating liver pool, and in V2, and the $^{13}$C-CE enrichments in V3. Examples of the $^{13}$C-incorporation curves are shown in Fig. 2.

As is indicated in Fig. 1A, the remaining parameters of the model include the five rate constants and V1, the pool of plasma FC and its rapidly equilibrating liver pool. The number of rate constants to be determined is reduced to three by application of the steady-state conditions, $k(2,1) = k(1,2) \times V_2/V_1$, and $k(3,1) = k(0,3) \times V_3/V_1$. Under these assumptions, the value of $k(1,2)$ is determined by the relation of the RBC enrichment to the FC enrichment, while $k(0,3)$ is determined by the relation of the CE enrichment to the FC enrichment. The SAAM-II program optimizes these parameters and the values of V1 and $k(0,1)$ to simultaneously fit the measured time evolution of the FC, RBC, and CE enrichments. The data for each subject was fitted separately.

The calculations of other parameters are summarized in the legend of Fig. 1A.

**TCE.** In Fig. 1A, cholesterol can leave the system either to the environment, represented by flux 1 = $k(0,1) \times V_1$, the rate constant for transfer of tracer from V1 to the environment (h$^{-1}$),...
RESULTS

Baseline characteristics

Seven carriers of a previously described mutation in APOAI (c.C643T, p.L202P) (19) and seven unaffected controls were included in this study. All 14 participants were males. Baseline demographic and lifestyle parameters, lipids, and lipoproteins of cases and controls are listed in Table 1. Plasma HDL-c and apoA-I concentrations of carriers were 63% and 43% lower than in controls (P = 0.001 and P < 0.008, respectively). No clinically manifest atherosclerotic cardiovascular disease was present in any of the participants. Plasma levels of triglycerides, apoB, FC, and CE were comparable in cases and controls (Table 1).

TCE

Figure 2 displays the 13C-incorporation curves of plasma FC, CE, and RBCs as sampled from a representative carrier and control participant during the 20 h infusions of 13C-C. There were no significant changes in plasma concentrations of FC or CE during the 13C-C infusions. The results of SAAM-II kinetic modeling are shown in Table 2. TCE was on average 19% lower in carriers compared with controls: 4.6 ± 0.79 mg/kg/h versus 5.7 ± 0.71 mg/kg/h, P = 0.02 (Table 2, Fig. 3). This difference retained statistical significance upon adjustment for age and BMI in linear regression analysis (P = 0.017).

In both groups, no significant correlations were observed between plasma concentrations of HDL-c and apoA-I and TCE: for carriers, β for HDL-c 0.40, P = 0.37, β for apoA-I 0.13, P = 0.78; for controls, β for HDL-c −0.37, P = 0.937, β for apoA-I 0.57, P = 0.19; for scatter plots of the individual data, see supplementary Fig. I. Flux 2, the FC exchange flux with the RBC FC pool, did not significantly differ between carriers and controls, nor did any of the other kinetic parameters (Table 2).

FSE

Carriers did not differ from controls when assessing the excretion of NSs and BAs in feces, as well as the percentage 13C recovery in fecal sterols and BAs (Table 3). Considerable intra-day as well as intersubject variability were observed. None of the fecal parameters were significantly correlated with plasma HDL-c levels in either group (supplementary Fig. II).

DISCUSSION

In this study we demonstrate that in vivo TCE is attenuated in patients with genetically determined low HDL-c. Carriers of mutations in APOAI with on average a 63% decrease in HDL-c levels as compared with controls, displayed a significant 19% reduction in TCE. This suggests that HDL-c contributes to the centripetal transport of cholesterol from peripheral tissues in humans. Clearly, this relation was not proportional indicating compensatory over efficient efflux by the remaining HDL particles or contribution of non-HDL-mediated RCT routes. The residual TCE and unaffected FSE in our patients with very low...
TABLE 1. Demographic parameters, plasma lipids, and lipoproteins

| Parameters                  | Carriers (n = 7) | Controls (n = 7) | P      |
|-----------------------------|-----------------|-----------------|--------|
| Age (years)                 | 44.7 (14)       | 39.3 (15)       | 0.49   |
| Number of men (%)           | —               | 7 (100)         | —      |
| BMI (kg/m²)                 | 29.8 (9.3)      | 25.1 (3.8)      | 0.24   |
| Number of smokers (%)       | 1 (14)          | 0 (0)           | 0.30   |
| Alcohol use (U/week) *      | 10 (8–13)       | 10 (8–13)       | 0.55   |
| Statin use (%)              | 0 (0)           | 0 (0)           | 0.30   |
| Total cholesterol (mg/dl)   | 167 (37)        | 183 (35)        | 0.41   |
| LDL-c (mg/dl)               | 112 (34)        | 110 (34)        | 0.91   |
| HDL-c (mg/dl)               | 20 (19)         | 54 (11)         | 0.001  |
| Triglycerides (mg/dl) *     | 129 (90–351)    | 101 (48–154)    | 0.14   |
| ApoA-I (mg/dl)              | 71 (39)         | 125 (22)        | 0.008  |
| ApoB (mg/dl)                | 95 (24)         | 77 (23)         | 0.18   |
| CE (mg/dl)                  | 45.6 (13)       | 42.2 (18)       | 0.94   |
| CEs (mg/dl)                 | 96.5 (16)       | 124 (48)        | 0.12   |

Data are presented as means (SD), or as a percentage of the total group. P values are for unpaired Student’s t-test on continuous variables, or for Chi-square test on categorical variables.

*Median and interquartile range. Because of skewed distribution, data on alcohol and triglycerides were log-transformed prior to testing.

HDL-c levels likely indicate that non-HDL pathways compensate and/or contribute significantly to TCE and fecal elimination of cholesterol in humans. Upregulation of these compensatory pathways in our patients with genetically low HDL-c may have led to underestimation of HDL-mediated TCE capacity.

Due to biological and methodological complexity, few studies to date have successfully addressed tissue cholesterol fluxes in man. The stable isotope infusion method used in the present study was shown to reproducibly measure TCE in both animals (28) and humans (18). Here a three-compartmental SAAM-II model was applied to optimally fit plasma 13C cholesterol enrichment data in order to calculate TCE, as well as the exchange flux of plasma FC with erythrocytes (27). Measurement of TCE as postulated in RCT requires its independence from net hepatic cholesterol flux into plasma, which in its turn requires successful equilibration of the infused 13C-C tracer with hepatic cholesterol (3). Although we could not determine this equilibration in our study, studies in bile fistula patients, allowing for direct measurement of tracers in bile, demonstrated that equilibration of FC within plasma lipoproteins and hepatic cholesterol pools occurs within hours (27). This study also indicated that the turnover of apoB-containing lipoproteins is at least one order of magnitude greater than the turnover of HDL particles (27). The rate of cholesterol equilibration will therefore mainly be determined by levels of apoB-containing lipoproteins; a decrease in HDL as present in the APOA1 L202P heterozygotes will not be of major influence on the rapid equilibration of FC.

TABLE 2. Kinetic parameters and cholesterol fluxes

| Parameters                  | Carriers (n = 7) | Controls (n = 7) | P      |
|-----------------------------|-----------------|-----------------|--------|
| Input                       |                 |                 |        |
| R (mg/kg/h)                 | 0.093 (0.02)    | 0.095 (0.01)    | 0.97   |
| V2 (mg/kg)                  | 42 (8.3)        | 36 (3.7)        | 0.12   |
| V3 (mg/kg)                  | 40 (20)         | 52 (6.9)        | 0.14   |
| Output                      |                 |                 |        |
| V1 (mg/kg)                  | 70 (29)         | 75 (12)         | 0.58   |
| k(0,1) (h⁻¹)                | 0.059 (0.015)   | 0.065 (0.016)   | 0.46   |
| k(0,3) (h⁻¹)                | 0.017 (0.002)   | 0.017 (0.002)   | 0.82   |
| k(1,2) (h⁻¹)                | 0.60 (0.39)     | 0.79 (0.33)     | 0.35   |
| k(3,1) (h⁻¹)                | 0.009 (0.003)   | 0.012 (0.004)   | 0.18   |
| Flux 1 (mg/kg/h)            | 3.9 (0.75)      | 4.8 (0.79)      | 0.06   |
| Flux 2 (mg/kg/h)            | 25.3 (14)       | 28.4 (12)       | 0.49   |
| Flux 3 (mg/kg/h)            | 0.66 (0.31)     | 0.88 (0.18)     | 0.11   |
| TCE (= flux 1 + flux 3) (mg/kg/h) | 4.6 (0.79) | 5.7 (0.71)     | 0.018  |

Kinetic input parameters were calculated as described in the Methods section. Kinetic output parameters and fluxes were calculated by curve-fitting of plasma FC, CE, and RBC FC 13C-enrichment curves using the SAAM-II model as described in Fig. 1A. Data are presented as means (SD). TCE was significantly lower in carriers compared with controls (P for t-test 0.018; difference independent of age, BMI, and flux 1 tended to be lower in carriers compared with controls (P for t-test = 0.06). None of the other kinetic parameters differed significantly between carriers and controls. R, infusion rate; V1, pool size plasma FC and rapidly equilibrating liver pool; V2, RBC FC pool size; V3, plasma CE pool size; k(0,1), transfer rate constant for tracer from V1 to environment; k(0,3), transfer rate constant for tracer from plasma CE pool to environment; k(3,1), transfer rate constant for tracer from V1 to plasma CE pool; k(1,2) transfer rate constant for tracer from RBC FC pool to V1; flux 1, flux of V1 to the environment; flux 3, flux of V1 to plasma CE pool; flux 2, exchange flux between V1 and RBC FC.
In vivo cholesterol efflux in HDL deficiency

studies, suggesting that apoA-I drives the first step of RCT. Plasma of apoA-I-deficient mice (29) and humans (30, 31) had decreased capacity to accept cholesterol from a stable cell line compared with control plasma. Also, apoA-I-deficient mice displayed a reduced \(^3\)H-cholesterol flux from intraperitoneal injected \(^3\)H-cholesterol-loaded macrophages through plasma toward the liver and into the feces (29, 32). However, these results have been disputed by other studies in apoA-I-deficient mice, indicating that apoA-I does not determine centripetal cholesterol flux to either the liver or the feces (12, 13, 33).

TCE was significantly reduced in carriers, but by only a third of the reduction in HDL-c, implying residual capacity and recruitment of compensatory mechanisms for TCE in humans with genetically low HDL-c. Tissue-derived cholesterol fluxes may in part be diverted via LDL-c that might take over the acceptor role in low-HDL states, although the question remains how these large apoB-containing particles reach the peripheral tissues that efflux FC. In future studies, we will assess in vivo cholesterol fluxes by the present method in patients with familial hypobetalipoproteinemia. These studies will likely provide answers to this matter. Alternatively, ABCG1 might compensate and promote cholesterol efflux onto HDL particles. Interestingly, the activity of ABCG1 does not influence overall HDL levels (34, 35), possibly, indeed, explaining the discrepancy between the magnitudes of reduction in HDL-c and in TCE observed in the carriers.

Of note, our kinetic model also permitted calculation of whole-body esterification. This LCAT-mediated step is net unidirectional (36) and was originally postulated as a central element in the RCT concept, as the driving force for TCE (37). However, we found esterification fluxes similar to those previously found in healthy controls (25). Interestingly, plasma cholesterol esterification fluxes did not differ between cases and controls, indicating cholesterol esterification independent of plasma apoA-I levels. In line, LCAT gene therapy has been demonstrated to correct low HDL-c levels in mice with mutations in \(\text{APOA1}^{1202P}\) (38). Previous studies in humans have also suggested that plasma CE clearance is mostly mediated by apoB100-containing particles following CETP-mediated transfer, rather than by direct HDL-dependent CE removal (13, 39, 40). Our

![Figure 3](Fig_3.png)

**Fig. 3.** TCE in \(\text{APOA1}^{1202P}\) carriers and unaffected controls. TCE (mg/kg/h) was calculated as the sum of flux 1 and flux 3 (Figure 1A). \(*\ast P\) value for univariate analysis (unpaired Student’s \(t\) test). The observed difference was statistically independent of age and BMI (\(P\) after adjustment for age and BMI: 0.017).

| TABLE 3. FSE and \(^{13}\)C-recovery |
|-------------------------------------|
| Fecal Excretion | Carriers of Mutations in \(\text{APOAI}\) (n = 7) | Controls (n = 7) | \(P\) |
| NSs (mg/day) | 2,015 (1,431) | 1,456 (404) | 0.43 |
| \(^{13}\)C recovery in NSs (%) | 18.2 (17) | 10.9 (5.8) | 0.50 |
| Fecal BAs (mg/day) | 607 (515) | 484 (218) | 0.57 |
| \(^{13}\)C recovery in fecal BAs (%) | 3.1 (3.4) | 2.3 (1.6) | 0.55 |
| Total fecal \(^{13}\)C recovery (%) | 21.3 (20) | 13.3 (6.3) | 0.33 |

Data are presented as means (SD) during 7 day fecal collection period postinfusion. \(P\) values are for unpaired Student’s \(t\) test.
A key observation raises the question whether patients with a genetic LCAT deficiency display a reduced TCE; the current model could serve as a means to investigate this. FSE measured by mass excretion of NSs and BAs as well as by $^{13}$C recovery in these fractions, was not significantly different between carriers and controls. In humans, equi- 

vocal data exist on the relation between HDL-c and FSE, showing decreased (15) as well as unaffected (41–43) FSE in relatively small populations with genetically determined low HDL-c levels. Another study in 63 healthy males even reported a negative correlation between HDL-c levels and FSE (30). Mouse studies have reported absence of a relation between HDL-c and FSE. ApoA-I-deficient mice were found to have normal FSE (13), and hepatobiliary cho- 

lesterol secretion and FSE were unaffected in ABCA1-deficient mice (11, 44). Furthermore, upregulation of individual steps in the RCT pathway did not affect FSE in mice (10). HDL intervention studies in man provide a mixed picture: although infusion of pro-apoA-I or rHDL increased FSE in four (16) and six (17) subjects, respectively, doubling HDL-c levels by CETP inhibition had no effect on FSE in 16 hypercholesterolemic individuals (45).

Of note and as observed in other studies (15, 16), the FSE data showed considerable variation, thus limiting their discriminative power. Yet the fecal $^{13}$C recovery spec- 

ifically shows that fecal loss of plasma-derived cholesterol is not primarily determined by plasma HDL-c levels, implying that other mechanisms contribute to whole-body cho- 

lesterol elimination. For instance, direct transintestinal cholesterol excretion (TICE) has been shown to contribute substantially to FSE in mice (46, 47). This nonbiliary route of sterol excretion was shown to be largely indepen- 

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dent of HDL as a cholesterol donor, as demonstrated in studies using ABCA1−/− and SR-B1−/− mice (46). Early in- 

testinal perfusion studies imply the presence of TICE in humans (48). In support of this concept, we recently re- 

ported that TICE may contribute significantly to FSE in humans, independently from apoA-I/HDL (49).

Two aspects merit closer consideration. First, our study does not provide proof of causality for the observed association between HDL-c and TCE. This would require fur- 

ther studies with apoA-I substitution. Also, our measurement of TCE and FSE does not quantify the efflux of cholesterol from the lipid laden macrophage via ABC-transporters to HDL for subsequent elimination into the feces (3, 7), as cholesterol in atherosclerotic plaques represents merely a minute fraction of the total exchangeable cholesterol pool size (21, 50). Assessment of this macrophage-specific RCT is currently confined to studies in mice (3).

In conclusion, carriers of a mutation in APOA1, charac- 

terized by strongly reduced plasma HDL-c levels, present with a reduced TCE compared with unaffected controls. This association strengthens the concept of apoA-I as a contributor to RCT, considered the main atheroprotective mechanism of HDL. Indeed, increased atherosclerosis was found in carriers of mutations in APOA1 (19). This first demonstration of the contribution of HDL-c to the cen- 

tripetal transport of cholesterol from peripheral tissues in humans holds promise for therapies aiming at an increase of plasma HDL-c levels and provides the means for in vivo assessment of the efficacy of RCT enhancing strategies.

The authors thank all participants for their participation.

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