# Effect of labeling of plasma lipoproteins with [$^3$H]cholesterol on values of esterification rate of cholesterol in apolipoprotein B-depleted plasma

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**Abstract The fractional esterification rate of cholesterol in apolipoprotein B (apoB)-depleted plasma ($FER_{HDL}$) is a good indicator of particle size distribution in high density lipoprotein (HDL) and low density lipoprotein (LDL). However, there has been a discrepancy in the absolute values of $FER_{HDL}$ published by different laboratories. Because the main difference between the methods was in the labeling of lipoproteins with [$^3$H]cholesterol we investigated the effect of using Corning immunoplates and paper discs as carriers of the labeled unesterified cholesterol. We found that Corning plates trap some $^3$H-labeled free cholesterol, which is released during incubation at 37°C. This means that this additional $^3$H-labeled free cholesterol is exposed to lecithin: cholesterol acyltransferase (LCAT) for a shorter time and artificially decreases $FER_{HDL}$. Using paper discs discarded before incubation as carriers of the $^3$H-labeled free cholesterol results in complete labeling of HDL and thus yields higher values of $FER_{HDL}$.**—Dobiášová, M., L. Adler, T. Ohta, and J. Frohlich. **Effect of labeling of plasma lipoproteins with [$^3$H]cholesterol on values of esterification rate of cholesterol in apolipoprotein B-depleted plasma. *J. Lipid Res.* 2000.** 41: **1356–1357.**

**Supplementary key words** [$^3$H]cholesterol labeling of lipoproteins • LCAT • cholesterol esterification rate in apoB-depleted plasma • $FER_{HDL}$

We have previously demonstrated that the fractional esterification rate of cholesterol in apolipoprotein B (apoB)-depleted plasma ($FER_{HDL}$) reflects the particle sizes of plasma high density lipoprotein (HDL) and low density lipoprotein (LDL) (1–5). This results from the fact that the smaller lipoprotein particles are better substrates for plasma lecithin:cholesterol acyltransferase (LCAT) than the larger particles. However, there has been a discrepancy in the absolute values for this parameter between our laboratories. For example, in children and in patients with coronary artery disease (CAD), the $FER_{HDL}$ values reported differed by more than 100% (5–8).

The measurement of $FER_{HDL}$ is based on estimation of the radioactivity of free and esterified cholesterol in plasma depleted of apoB lipoproteins (HDL-plasma), labeled with $^3$H-labeled free cholesterol at 4°C, and then incubated for 30 min at 37°C. Under these conditions only the free cholesterol on HDL is a substrate for lecithin:cholesterol acyltransferase (9).

We compared different labeling methods, using EDTA plasma depleted of apoB lipoproteins by precipitation with phosphotungstate and $MgCl_2$ (10); HDL-plasma from 21 subjects was used in three experiments.

In the first experiment (DISCS) we used our standard procedure (9). Paper discs containing homogeneously dispersed [$^3$H]cholesterol (0.075 μCi) were immersed in diluted HDL-plasma (100 μL of Tris-saline buffer and 50 μL of HDL-plasma in glass tubes on ice). By this procedure HDL is homogeneously labeled with [$^3$H]cholesterol, transferred from discs after 18 h of incubation at 4°C. The discs are then removed and the tubes placed in a shaking water bath for 30 min at 37°C.

In the second experiment (CORNING 1) tissue culture plates were used for labeling as described by Ohta et al. (11): here the [$^3$H]cholesterol was incorporated onto polystyrene tissue culture wells (Corning, Acton, MA). Absolute ethanol (100 μL) containing 0.2 μCi of [$^3$H]cholesterol was placed in the wells and then dried off by flushing with nitrogen. One hundred μL of plasma depleted of apoB lipoproteins in 400 μL of phosphate-buffered saline was added to each well and [$^3$H]cholesterol was equilibrated with the unesterified cholesterol of each sample by incubation at 4°C for 16 h. The Corning plates were then

Abbreviations: apo, apolipoprotein; CAD, coronary artery disease; FER, fractional esterification rate; HDL, high density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoprotein; TLC, thin-layer chromatography.

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TABLE 1. $^3$H radioactivity in 10-μL samples before and after 30-min incubation at 37°C and $FER_{HDL}$ in the three experiments

| | DISCS | | CORNING 1 | | CORNING 2 | |
|---|---|---|---|---|---|---|
| | Before | After | Before | After | Before[a] | After |
| dpm | 5645 ± 796 | 5957 ± 507 | 956 ± 393 | 1869 ± 643 | 956 ±3 93 | 988 ± 399 |
| $FER_{HDL}$ | | 19.55 ± 9.82 | | 10.22 ± 5.13 | | 19. 20 ± 8.47 |

Values given as means ± SD.
[a] Same samples as in CORNING 1.

transferred into a shaking water bath and incubated for 30 min at 37°C.

A third experiment was carried out to assess the effect of the Corning polystyrene tissue culture wells (CORNING 2): The procedure was the same as in CORNING 1 but before the incubation at 37°C the labeled samples of HDL-plasma were transferred from the Corning plates to new glass tubes and processed as described above.

In all three experiments lipid extracts were dried under nitrogen, and unesterified and esterified cholesterol was separated by thin-layer chromatography (TLC) and detected with iodine vapors. The resulting spots were cut from the plates, transferred into scintillation vials, and counted (9).

Radioactivity was measured in samples before and after a 30-min incubation at 37°C to determine whether there was additional flux of [$^3$H]cholesterol from the Corning wells (CORNING 1). Results shown in **Table 1** confirm that the polystyrene material of the Corning plates trapped some $^3$H-labeled free cholesterol and that it was released during incubation at 37°C. Thus some of the $^3$H-labeled free cholesterol was not utilized during the entire 30 min of incubation.

$FER_{HDL}$ values were significantly different between DISC and CORNING 1 experiments but were similar in DISCS and CORNING 2 experiments. This means that [$^3$H]cholesterol released from the Corning wells during the incubation (and not used for the LCAT reaction) artificially decreases $FER_{HDL}$ (Table 1).

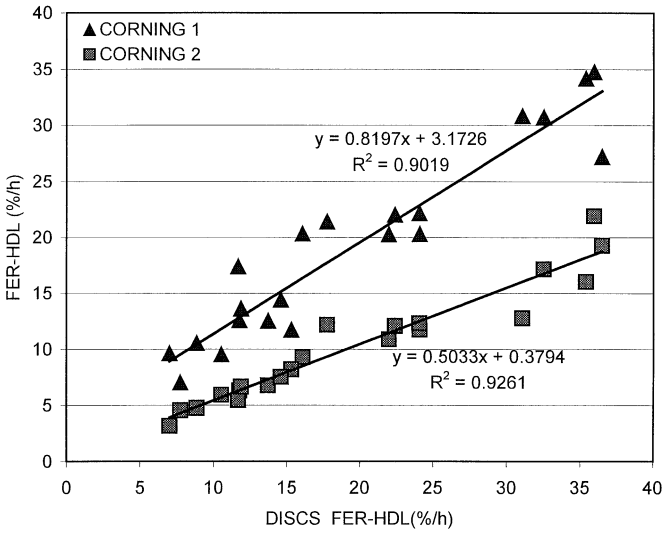


**Fig. 1.** Scatterplot showing relationships between $FER_{HDL}$ measured in DISCS and in CORNING 1 and 2 experiments.

However, the $FER_{HDL}$ values, from both CORNING 1 and 2 experiments are highly correlated with the DISCS values (**Fig. 1**).

In conclusion, the trapping of labeled free cholesterol on Corning plates explains the differences in absolute values of $FER_{HDL}$ between the two laboratories.

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