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Research Article

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Evaluation of Pathways for the Cellular Uptake of High Density Lipoprotein Cholesterol Esters in Rabbits

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Abstract

Cholesterol esters (CE) formed in HDL by lecithin:cholesterol acyltransferase are thought to mediate the return of cholesterol from extrahepatic tissues to the liver for excretion or reutilization. Several pathways may be involved in that process. Tracer kinetics were used to estimate the contributions of the various pathways to cellular uptake of HDL CE in rabbits. Tracers of HDL CE, HDL apo A-I, LDL apo B, and VLDL CE were simultaneously injected intravenously. Plasma decays were followed for 24 h in 4 lipoprotein pools: HDL without apo E, HDL with apo E, LDL, and VLDL. Kinetic analysis of the resulting plasma decay curves revealed that the preponderance of plasma CE (> 90%) originated in the HDL fraction. About 70% of HDL CE were cleared from plasma after transfer to LDL and VLDL, 20% were cleared directly from the HDL pool without HDL particle uptake ("selective" uptake), and 10% were cleared in HDL particles (including particles containing apo E). Since rabbits have about four times the plasma cholesterol ester transfer activity of man, and since the transfer pathway must compete with the selective uptake pathway, these results make it likely that selective uptake plays a substantial role in humans in the clearance of HDL CE. (*J. Clin. Invest.* 1991. 87:331–346.) Key words: low density lipoproteins • very low density lipoproteins • selective uptake • cholesterol ester transfer protein • reverse cholesterol transport

Introduction

It is widely accepted that HDL plays a central role in the return of "excess" cholesterol from extrahepatic tissues to the liver for excretion or reutilization (1, 2). HDL, and perhaps other lipoproteins, accept cholesterol from cells. Some part of this cholesterol is esterified by the lecithin:cholesterol acyltransferase system in the HDL fraction, which sustains the acceptance of cell cholesterol through its removal to the neutral lipid core. What then happens to the resulting HDL cholesterol esters is not clear, and several pathways almost surely play a role in their eventual removal from the circulation.

A pathway widely thought to dominate in man involves plasma cholesterol ester transfer protein (CETP)[1] (1, 3). CETP mediates the net transfer of HDL cholesterol esters to more buoyant lipoproteins in exchange for triglycerides (4), and these more buoyant lipoproteins are then taken up mostly by the liver; the triglycerides transferred to HDL are hydrolyzed intravascularly, allowing HDL to begin the cycle again. Such a role for CETP in rabbits is supported by studies in which CE transfer was blocked using antibodies against CETP (5), which indicated a substantial role for CETP in clearance of HDL cholesterol esters. A second pathway proposed to play a role in HDL cholesterol ester uptake involves apo E (6, 7). According to this hypothesis, cholesterol esters accumulate in HDL due to the action of lecithin:cholesterol acyltransferase; the enlarged HDL particles progressively acquire apo E at the expense of apo A-I, and the apo E targets them for hepatic uptake. As a corollary to this hypothesis, it is also possible that apo E-rich HDL particles arise independently of the general HDL pool and mediate return of cholesterol esters to the liver. Of course, HDL particles can also be taken up without benefit of apo E; in humans, the rate of clearance of HDL apoproteins is so low as to indicate that this route is a small contributor to total HDL cholesterol ester flux (8). Finally, HDL cholesterol esters may be directly taken up without uptake of the HDL particles, a process we designate as "selective uptake" (9, 10). This pathway is responsible for the majority of HDL cholesterol ester uptake by the liver of rats (9, 10).

The studies mentioned above, as well as others not cited, have given some idea of the roles of the various pathways for metabolism of HDL cholesterol esters in some animals. However, if we are to understand the relative importance of those pathways and their interactions in any animal, it is necessary to measure all of the competing pathways in that animal. We undertook to make such simultaneous measurements in rabbits. The choice of rabbits was based largely on our interest in the selective uptake pathway, and the hypothesis that the pathway plays a significant role in clearance of HDL cholesterol esters in humans. The rationale was that if we could discern the selective uptake pathway in rabbits, which by our assays (11) have about four times the plasma CETP activity of humans toward endogenous substrates and thus presumably a more rapid rate of uptake by the competing CETP-mediated pathway, then the selective uptake pathway is likely to play a role in man as well. Rabbits indeed have the potential to express the pathway in vivo, as shown by selective uptake of HDL cholesterol esters by cultured fibroblasts from normal and LDL receptor-deficient rabbits at rates comparable to those of human

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1. *Abbreviations used in this paper:* apo, apolipoprotein; CETP, cholesterol ester transfer protein; ^{125}I-NMTC-apo A-I, apo A-I derivatized with the radioiodinated *N*-methyl tyramine cellobiose ligand; ^{131}I-TC-LDL, LDL derivatized with the radioiodinated tyramine-cellobiose ligand.

fibroblasts (Goldberg, D. I., and R. C. Pittman, unreported results).

Examination of lipoprotein cholesterol ester metabolism in animals with plasma CETP activity is complicated by the rapid movement of cholesterol esters between lipoprotein pools. It is not possible to directly determine the pool from which the cholesterol ester tracer is removed, and kinetic analysis of the exchange and transfer reactions is necessary to determine the rate of removal from any of the pools. To carry out such a kinetic analysis, we made use of multiple lipoprotein tracers that were simultaneously administered; intracellularly trapped tracers (12) were used as much as possible to minimize tracer recycling. The tracer contents of multiple lipoprotein pools were followed for 24 h, and the plasma decay curves were analyzed by compartmental analysis.

The results of these studies indicate that, as expected, the major pathway for clearance of HDL cholesterol esters in rabbits is the CETP-mediated "transfer pathway". However, selective uptake also played an important role in most animals. The removal of cholesterol esters in HDL particles made a minor contribution to overall clearance of HDL cholesterol esters, and cholesterol ester uptake from HDL with apo E was not discernibly different than removal from the HDL pool without apo E.

Methods

Preparation of lipoprotein tracers. Lipoproteins were prepared from the plasma of overnight-fasted female New Zealand White rabbits by equilibrium density ultracentrifugation using conventional techniques (13). The VLDL fraction was prepared at $d < 1.02$ g/ml, LDL was prepared at $1.02 < d < 1.06$ g/ml, and HDL at $1.06 < d < 1.21$ g/ml. The HDL fraction was depleted of particles with apo E by heparin-agarose affinity chromatography before labeling (14).

Both ester and ether tracers of cholesterol esters were used. [^{14}C]-Cholesteryl-oleyl ether was prepared from [4-^{14}C]cholesterol (Amersham Corp., sp act 60–90 mCi/mmol) and [^{3}H]cholesteryl-oleyl ether was prepared from [7(n)-^{3}H]cholesterol (Amersham Corp., Arlington Heights, IL; sp act 5–15 Ci/mmol) in carrier-free reactions, as previously described (15, 16). Cholesterol-[1-^{14}C]oleate was purchased from Amersham Corp. (sp act 50–60 mCi/mmol). Cholesterol-[^{3}H]-oleate was prepared using [9,10(n)-^{3}H]oleic acid (Amersham Corp.; sp act 4.3 Ci/mmol). The acid anhydride was formed by reflux with acetic anhydride, and the purified anhydride was then reacted with excess cholesterol in the presence of dimethyl-aminopyridine (17). The product ester was purified by TLC, and its purity checked by HPLC.

Apo A-I was purified from rabbit HDL and derivatized with the *N*-methyl tyramine cellobiose (^{125}I-NMTC) ligand as previously described (10, 12, 16).

HDL was doubly labeled by incorporation of ^{125}I-NMTC-apo A-I and either cholesterol-[^{3}H]oleate or [^{3}H]cholesteryl-oleyl ether. The lipid tracer was first incorporated from a donor liposomal preparation (18) by CETP-mediated transfer, as previously described (16), and then reisolated at $d > 1.05$ g/ml. The ^{125}I-NMTC-apo A-I was then incorporated into the particles by exchange (free apo A-I mass < 10% of HDL protein), and the HDL was reisolated by flotation at $d < 1.21$ g/ml (16).

Cholesterol-[1-^{14}C]oleate or [^{14}C]cholesteryl-oleyl ether was incorporated into VLDL by transfer from synthetic HDL (19) containing the tracer. This process was catalyzed by CETP activity in the $d > 1.25$ fraction of rabbit plasma. The labeled VLDL was then reisolated by flotation at $d < 1.02$.

LDL was directly derivatized with the ^{131}I-tyramine-cellobiose ligand as previously described (16). This was done as shortly before injection into the rabbits as possible to minimize radiation damage; the preparation was usually completed no earlier than 24 h before use. Polyacrylamide gel electrophoresis disclosed that the ^{131}I comigrated predominantly with apo B (> 90%), but discernible peaks of activity were observed corresponding to apo E and apo A-I. Less than 4% of the tracer was extracted into 2:1 chloroform:methanol.

Turnover studies in rabbits. Female New Zealand White rabbits, 2.5–3.0 kg, were fasted overnight before study and then during the 24-h duration of the study. Tracer lipoproteins, prepared as outlined above, were mixed just before use and then injected into a medial ear artery. Injected doses were: $0.6–2.8 \times 10^6$ dpm for ^{14}C, $0.7–2.9 \times 10^6$ dpm for ^{3}H, $3.3–9.8 \times 10^6$ dpm for ^{125}I, and $1.3–11.1 \times 10^6$ dpm for ^{131}I. Injected mass of tracer was < 2% of the plasma pool for HDL and LDL, and < 10% for VLDL. Blood samples (usually 0.5 ml) were periodically withdrawn from a marginal ear vein into EDTA during the study. To minimize transfer of cholesterol ester tracer between lipoprotein pools after blood sampling, the samples were immediately chilled and plasma was quickly separated. The plasma was diluted to 5 ml with cold sucrose solution to a final density of 1.025 g/ml. These samples were kept refrigerated until all samples were collected, and all were simultaneously ultracentrifuged. Tests of this handling showed that doubly-labeled HDL added to blood and treated in this way was recovered almost completely in the HDL fraction (more than 90%); that small amount of HDL cholesterol ester tracer recovered in more buoyant fractions was in proportion to apo A-I tracer also recovered in these fractions, and was no more than found when the labeled HDL was added just before ultracentrifugation. This indicates that cholesterol ester tracer did not continue to exchange between fractions during sample analysis.

At termination of the study, a large blood sample (10–20 ml) was taken for lipoprotein fractionation and analysis of cholesterol and cholesterol esters.

At termination of the study of two rabbits, the vasculature was thoroughly perfused and tissue samples were taken for analysis of ^{125}I-NMTC-apo A-I uptake, as previously described (20).

Assays. Lipoproteins were separated from the periodically withdrawn blood sample into density classes by sequential ultracentrifugation of plasma samples (13), usually 200 μl. Pig plasma (1 ml) was added as carrier because of the small volume of rabbit plasma available; pig plasma has very low CETP activity (21). Sucrose was used to adjust densities since high salt concentrations interfered with the subsequent steps to separate HDL particles containing apo E. VLDL was prepared at $d < 1.02$ g/ml, LDL at $1.02 < d < 1.06$ g/ml, and HDL at $1.06 < d < 1.21$ g/ml. All centrifugations were carried out in 6-ml tubes in a Beckman 50.3 rotor (Beckman Instruments, Palo Alto, CA).

HDL particles with apo E were separated from the general HDL population by heparin affinity. In some experiments this was done by a heparin-manganese precipitation method (11, 22), and in others by adding plasma to heparin-agarose beads (23) which were then sedimented to remove the heparin-bound HDL particles. In the case of heparin-manganese precipitation, pig plasma was added as a carrier. The recovery of apo E in the apo E-rich fraction, as well as the overall recovery of apo E, varied between techniques and between experiments. However, in no case were the kinetics of the lipid tracers in this fraction clearly distinguishable from the kinetics of HDL without apo E. This was true even when the precipitation was most selective (the recovery of apo E incomplete, and the precipitated particles most enriched in apo E). On these grounds, the fraction of HDL containing apo E was not considered as a separate HDL pool in further modeling.

Cholesterol was analyzed by a fluorometric method (24), and cholesterol esters were determined as the difference in cholesterol level before and after ester hydrolysis. In some experiments, results from this method were compared to results from use of a cholesterol assay kit from Sigma Chemical Co. (St. Louis, MO) and to results determined by the Lipid Research Clinic at The University of California at San Diego according to their well standardized techniques. Results were reasonably comparable.

Kinetic analysis. Kinetic data were analyzed using multicompartmental modeling. All modeling was performed on a MicroVAX II computer (Digital Equipment Co., Maynard, MA) using the CONSAM (25) and SAAM (26) computer programs.

The model used is shown in Fig. 1. The starting model consisted of three compartments representing plasma pools of VLDL, LDL, and HDL with bidirectional cholesterol ester exchange pathways between each pair of compartments. The HDL compartment had two pathways for irreversible cholesterol ester removal: one via uptake of the whole particle as traced by ^{125}I; a second particle-independent pathway corresponding to the direct uptake of cholesterol esters from the HDL particle. A similar particle-independent cholesterol ester removal pathway was tried for LDL, but was found not to be identifiable. Our analyses therefore assumed that all cholesterol ester removal directly from the LDL compartment was via catabolism of the whole LDL particle at a rate determined primarily by the disappearance of ^{131}I. Since VLDL particles were not directly labeled, we could not discern uptake in VLDL particles from potential uptake by direct removal of cholesterol esters. The VLDL compartment therefore had a single removal route. The LDL and HDL compartments were each in exchange with extravascular compartments, as required by the biexponential decay of the apoprotein tracers.

All kinetic data for each rabbit were fit simultaneously and the model parameters adjusted by SAAM to give the best fit to all tracer data. The model solutions for the cholesterol ester tracers, ^{14}C and ^{3}H, differed only in the initial distribution of the label, all fractional transfer and degradation rates being equal for the two solutions. The model solutions for the apoprotein tracers had the intravascular exchange rates and particle-independent catabolic rates set to zero. The kinetics of ^{131}I-LDL were therefore determined solely by the rate of direct removal of LDL particles and the rates of exchange with the extravascular LDL compartment. The kinetics of ^{125}I-HDL were determined by the rate of particle-dependent degradation of HDL and the rates of exchange with the extravascular HDL compartment.

When this initial model was applied to the kinetic data, systemic errors were found in the fits to the tails of the VLDL ^{3}H and ^{14}C curves for all rabbits and in the tails of the HDL ^{3}H curves for about half the animals. Two additions to the model were therefore required.

First, an exchange pool for HDL cholesterol ester was added to fit the tail of the HDL ^{3}H decay curve. Exchange of cholesterol ester with this pool required removal of the lipid from the HDL particle since exchange of the whole particle was inconsistent with the ^{125}I data. The rate constants for this exchange had to be set to zero when fitting the ^{125}I data. The parameters for the HDL cholesterol ester exchange pool were found to be well defined (asymptotic coefficients of variation < 80%) in six of the rabbits. The weighted mean value of the fractional rate of transfer from the exchange compartment back into the plasma HDL compartment was 0.302 pool/h for these six animals. For each of the seven rabbits for which the exchange rates were initially poorly defined (asymptotic coefficients of variation > 100%), the fractional transfer rate from the extravascular compartment into the plasma compartment was fixed at 0.302, and the fractional rate from the plasma compartment into the extravascular compartment adjusted by SAAM. This technique allowed the calculation of an asymptotic standard deviation for the size of the exchange pool under the assumption that the residence time of cholesterol in that pool would be 3.3 h. As expected, the exchange pools for these rabbits, as determined by the transfer rates into the pool, were found to be small or nonexistent.

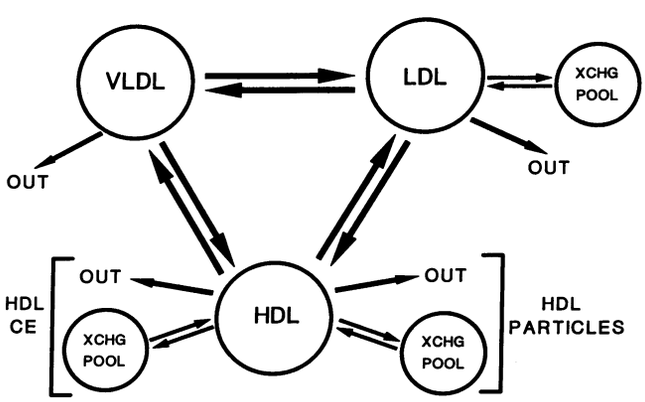

Figure 1. Compartmental model for the metabolism of plasma cholesterol esters in rabbits.

A pool of slowly catabolized VLDL particles was added to the initial model to fit the tail observed in all VLDL curves. This exponential component was fit using a slowly turning over plasma VLDL compartment rather than an extravascular exchange pool, because the latter model was inconsistent with the kinetic data, predicting similar slow components in all lipoprotein fractions. Such a slow exponential component was observed in neither the HDL nor the LDL data. A slow VLDL pool is also consistent with other models of VLDL metabolism in rabbits (27). The contribution of the slowly removed VLDL was small, and an average of only 4.3% of total VLDL cholesterol ester transport passed through this pathway. The slow VLDL pool is not shown independently of the general VLDL pool except in the appended detailed data.

Parameters determined for individual animals and experiments are shown in the appendix. Standard deviations shown for individual parameters are asymptotic standard deviations as calculated by SAAM (26). These error estimates correspond to coefficients of variation which are generally < 50%, indicating that the model parameters were indeed mathematically identifiable from the turnover data obtained. Standard deviations of mean parameter values shown in Results are for the population of estimated parameters and are independent of the asymptotic errors calculated by SAAM. These error estimates correspond to coefficients of variation which are in many cases > 100%, indicating large variability in the estimated model parameters. Inspection of the individual data in the appendix discloses that most of this variation lies between studies and that results within a single series of rabbits are more consistent.

Results

To examine the routes for removal of HDL cholesterol esters from plasma of intact rabbits, four plasma lipoprotein tracers were simultaneously administered to rabbits, and four plasma lipoprotein pools were examined during the 24-h period after administration. This yielded 12 useful plasma decay curves per animal which were then analyzed by compartmental analysis. The HDL fraction was labeled in its cholesterol ester moiety using either [^{14}C]cholesterol oleate or [^{14}C]cholesteryl-oleyl ether, and also in its apo A-I moiety using ^{125}I-NMTC-apo A-I. To constrain the modeling, LDL was labeled in its apo B moiety (^{131}I-tyramine cellobiose (TC)-LDL), and VLDL was labeled in its cholesterol ester moiety using [^{3}H]cholesterol oleate or [^{3}H]cholesteryl-oleyl ether. 3 sets of 3 or 4 rabbits (10 animals) were studied tracing cholesterol esters with radioactive cholesteryl-oleyl ethers, and 1 set of 3 rabbits was studied tracing the esters with radioactive cholesterol oleate.

The kinetics were modeled independently in each of the rabbits studied using all available data. The final model applied to the data is shown in Fig. 1 and described in Methods. Several features of the model should be noted here. The removal of HDL cholesterol esters concomitant with HDL particle uptake was constrained to be at the same fractional rate as removal of

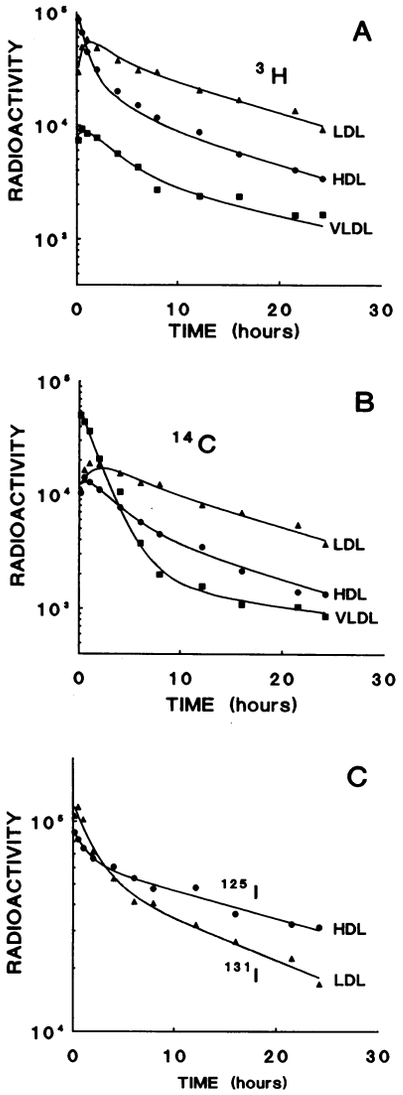

Figure 2. Data and computer-generated fits for the plasma decay of lipoprotein tracers. (*A*) Appearance of ^{3}H in lipoprotein pools resulting from the introduction of [^{3}H]-cholesterol oleate in HDL. (*B*) Appearance of ^{14}C resulting from the introduction of [^{14}C]cholesterol oleate in VLDL. (*C*) Decay of ^{125}I from the HDL pool that was labeled with ^{125}I-NMTC-apo A-I, and of ^{131}I from the LDL pool that was labeled with ^{131}I-TC-LDL.

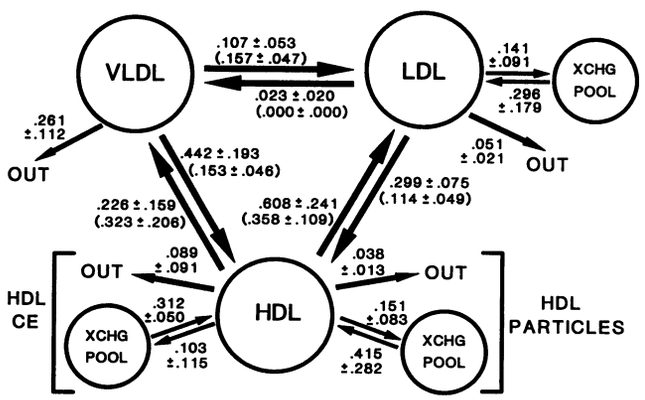

Figure 3. Fractional transfer rates for lipoprotein cholesterol esters in rabbits. Fractional rates (h^{-1}) for the various processes of the model in Fig. 1 are shown. Results for cholesterol ester and cholesterol ether tracers are shown independently for those processes dependent on CETP (intravascular transfers); results for the ether tracers are shown in parentheses. Results for ester and ether tracers are pooled for all other processes.

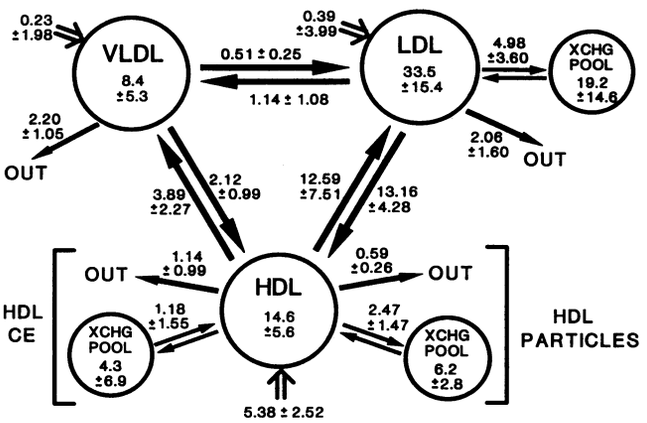

Figure 4. Mass transport rates for lipoprotein cholesterol esters in rabbits. Cholesterol ester mass fluxes (mg cholesterol/dl per h) for those processes dependent on CETP (intravascular transfers) were determined using only data from experiments using cholesterol ester tracers. Mass transports for all other processes were determined using data from all experiments. Cholesterol ester pool sizes (mg cholesterol/dl) are from all experiments. Vascular pool sizes were measured, and exchange pools were estimated.

the ^{125}I-apo A-I tracer. This assumes homogeneity of the HDL pool and that apo A-I traces the fate of HDL particles. The clearance of cholesterol esters from the LDL pool by uptake of LDL particles was similarly constrained to be at the same fractional rate as clearance of LDL apo B measured with ^{131}I-TC-LDL, which again assumes homogeneity. Extravascular exchange pools for HDL and LDL particles were included in the model. Whereas the kinetics of cholesterol ester removal from HDL particles with and without apo E were similar, all HDL particles were included in a single HDL pool to allow better definition of the other parameters. The irreversible removal of cholesterol esters from HDL without parallel removal of HDL apo A-I (selective uptake) was allowed, and was determined as the difference in the fractional rate of removal of cholesterol esters and apo A-I from the HDL pool; this parameter was defined in 13 of the 16 animals. In 6 of the 16 animals the data were better fit when it was allowed that there be an extravascu-

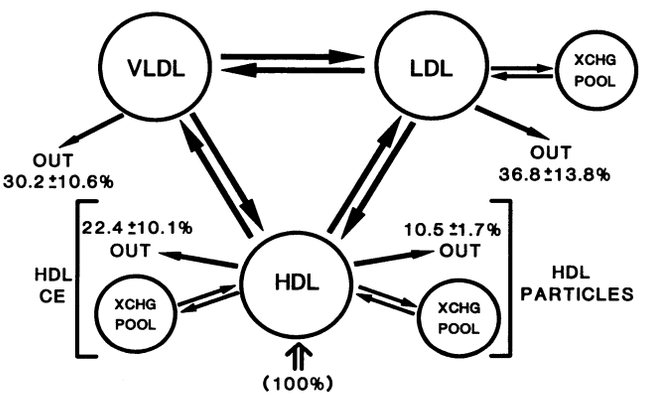

Figure 5. Routes of removal of HDL cholesterol esters as a percentage of cholesterol esters entering the HDL pool. Results are the average from only those rabbits that were studied using cholesterol ester tracers.

lar exchange pool for HDL cholesterol esters independent of HDL particles. This is in keeping with independent biological evidence for such an exchange pool in the plasma membrane of cultured cells (28). Based on evidence for selective uptake of cholesterol esters from LDL (29) (which we have verified but found to occur at a much lower rate than from HDL), a pathway for direct uptake of LDL cholesterol esters was tried in all animals and found to be negligible in all; it was not included in the final model.

Fig. 2 shows representative plasma decay data for the four tracers in a single animal, and the curves fitted to those data according to the model described above. This animal was deemed representative on the grounds that the determined parameters were nearest the means of all animals. The data are shown for each of the three lipoprotein pools. Fig. 2 *A* shows the data for cholesteryl-[^{3}H]oleate introduced into the HDL pool, and Fig. 2 *B* shows the data for cholesteryl-[^{14}C]oleate introduced into the VLDL pool. Fig. 2 *C* shows the decay of ^{125}I-NMTC-apo A-I from the HDL pool and ^{131}I-TC-LDL from the LDL pool; the small amount of these tracers in other pools is not shown but was accommodated in the modeling.

Fig. 3 shows the fractional rates determined according to the model of Fig. 1. Since the ether is not transferred by CETP at the same rate as the ester (11), the rate constants for intravascular transfer of ester and ether are shown separately. However, other studies have indicated that cholesteryl esters and ethers are similar with respect to other uptake processes (similar plasma decay kinetics in rats that lack CETP activity [10, 11], and similar in vitro uptake rates (10)); therefore, rates for both types of tracers were pooled in those cases.

The rates for CETP-mediated transfer of ester tracer and of ether tracer (in parentheses) differed, and the ester was generally transferred at a greater rate than the ether, in agreement with the difference in transfer rates from HDL to LDL determined in vitro (11). The transfer of cholesterol esters from VLDL to LDL included the movement of VLDL particles into the LDL pool; the small rate of reverse transfer from LDL to VLDL (which was poorly defined in most animals) suggests that there was little CETP-mediated transfer between these pools and that particle transfer accounted for most of the transfer from VLDL to LDL. The transfer rates between the HDL and VLDL pools, and between the HDL and LDL pools, were both much higher than the LDL-VLDL rates.

Fig. 3 also shows the fractional rates of transfer of cholesterol esters for processes that are not CETP dependent. Previous studies have shown no apparent difference between ester and ether in these processes (9–11) and this study did not indicate a difference. The rate designating selective uptake ("out" for "HDL CE") was determined as the rate of irreversible removal of cholesterol esters from HDL minus the rate of removal of HDL particles as determined using ^{125}I-NMTC-apo A-I. This does not account for any apo A-I that may be cleared independently of HDL particles. In rats, clearance of free apo A-I by the kidney accounts for a major fraction of total apo A-I clearance (10), but this appears not to be a the case in rabbits; the tissues of two animals were examined at termination of the experiment for ^{125}I resulting from uptake of ^{125}I-NMTC-apo A-I, and kidney was found to account for only 5.5 and 6.1% of total recovered ^{125}I. Thus, clearance of free apo A-I by kidney apparently did not lead to an important underestimate of whole-body selective uptake.

Fig. 4 shows the modeling results in terms of mass transports through the various pathways, which takes into account the measured lipoprotein cholesterol ester masses. The study which used cholesterol ester tracers and two of the three studies which used cholesteryl ether tracers were considered; cholesterol ester masses were not measured in the third ether study. The rate constants and pool sizes for all of these animals (10 rabbits) were used to determine transports that were not CETP dependent, while only those animals traced with esters were used to determine those transport rates dependent on CETP (3 rabbits). Fig. 4 also shows the measured pool sizes and the calculated transport rates for all parameters of the model. These results indicate that plasma cholesterol esters were cleared mostly by uptake of VLDL and LDL particles and by selective uptake from HDL, with a smaller contribution from clearance of HDL particles (which includes particles with apo E). Clearance from the LDL pool accounted for 34% of total plasma cholesterol ester, clearance from the VLDL pool accounted for 37%, clearance by selective uptake from HDL for 19%, and clearance in HDL particles for 10%. The CETP-mediated flux between lipoprotein pools was high relative to the rate of irreversible removal of cholesterol esters from any pool. This transfer was predominantly by exchange reactions. However, the flux of cholesterol esters from HDL to VLDL was greater than the return flux, indicating a net transfer of cholesterol esters to VLDL (2.95±2.24 mg ester cholesterol/dl per h for all rabbits). The flux of cholesterol esters between the HDL and LDL pools was large and dominated by exchange of esters with little evidence for net transport. Considering all studies, there was a minor and highly variable net transport from the HDL pool to the LDL pool (0.69±2.40 mg ester cholesterol/dl per h for all rabbits). If real, this modest net transfer would be consistent with the relatively high triglyceride content of rabbit LDL and with evidence that net transfer of cholesterol esters occurs in exchange for triglycerides.

Another salient conclusion from the parameters of Fig. 4 relates to the origin of lipoprotein cholesterol esters. It is evident that the input of cholesterol ester mass into plasma was predominantly into the HDL pool, and little ester was secreted in VLDL. This supports the conclusion of others that plasma cholesterol esters arise predominantly from the action of lecithin:cholesterol acyltransferase (30), and that the product esters enter first into the HDL pool (31). Since this result is in agreement with a large body of independent studies, the result also tends to support the validity of the modeling results presented here.

As an alternative description of the data, we determined the contribution of each pathway to clearance of HDL cholesterol esters as a fraction of cholesterol esters entering into the HDL pool. This estimation depends only on the determined rate constants and is independent of measured cholesterol ester masses. Using this approach, the experiments with tracer cholesteryl ether should underestimate the CETP-mediated pathways and overestimate selective uptake. Indeed, the data for animals traced with ether and ester differed in this direction. Consequently, only results for the animals traced using cholesterol ester are shown. The means of the individually determined transports of cholesterol ester mass originating in the HDL pool are shown in Fig. 5. The data are normalized to the input of cholesterol ester mass into the HDL pool, which is arbitrarily set to 100%. It is directly evident from this figure that

about two-thirds of HDL cholesterol esters are irreversibly removed from the plasma after transfer to more buoyant lipoproteins, with about equal amounts cleared from the VLDL and LDL pools. The direct removal of HDL cholesterol esters independent of HDL particles (selective uptake) accounted for ~ 22% of total clearance. The irreversible clearance of HDL particles, determined in terms of ^{125}I-NMTC-apo A-I, accounted for only 10% of total HDL cholesterol ester clearance.

Discussion

The purpose of this study was to quantify the various pathways for HDL cholesterol ester uptake in a model animal, from which the likely importance of these pathways in humans might be inferred. One central objective was to examine the probability that selective uptake plays a role in HDL metabolism in man. This determined the choice of rabbits for study because they might be expected to express more vigorously than man the pathway that must compete with selective uptake, the CETP-mediated transfer of cholesterol esters to more buoyant lipoproteins which are then taken up by endocytotic pathways. If selective uptake were found to play a role in rabbits, it seemed very likely that the pathway would play a role in humans as well.

The CETP-mediated pathways were accentuated in this study and selective uptake minimized not just by the choice of rabbits as the study subject, but also by the choice of cholesterol ester tracers. Cholesterol oleate traced plasma cholesterol esters, while the linoleate ester is the major plasma ester in rabbits (32). The oleate ester is both more rapidly transferred by CETP than the linoleate ester (33), and selectively taken up at a slower rate; in our hands cholesterol linoleate is transferred from HDL to LDL at 0.8 times the rate of oleate (in an all human system), but is selectively taken up by mouse adrenocortical tumor cells at 1.3 times the rate of oleate (Green, S. R., and R. C. Pittman, unreported results). On these grounds the rabbit studies reported here would be predicted to give a minimum estimate of the role selective uptake might play in humans, although differences in lipoprotein concentrations and compositions, as well as other factors, surely alter the equation.

In spite of an experimental design that emphasized the transfer pathway and mitigated against observing the selective uptake pathway, selective uptake was still defined in most rabbits. On average, the pathway accounted for ~ 19% of the total plasma flux of cholesterol esters and 22% of clearance of HDL cholesterol esters. However, the results were variable, and selective uptake accounted for as much as 47% or as little as none of the total HDL cholesterol ester cellular uptake. The reasons for this unanticipated variability are not clear, but some likely explanations are apparent.

First, it should be recognized that the variability was not somehow a consequence of the modeling process. Initial parameter estimates used in the final modeling were the same for all animals, and coefficients of variation for the determined parameters were generally < 50%. This indicates that the model parameters were indeed mathematically identifiable from the turnover data obtained. In contrast, comparing parameter values between animals disclosed coefficients of variation that were in many cases greater than 100%, and much of this variation was interexperimental. This suggests that some factor that varied between experiments, such as metabolic status or properties of the lipoprotein tracers, explained much of the variability.

One such factor that might vary between experiments and animals is the level of CETP activity. It might be expected that plasma CETP activity should correlate negatively with the selective uptake of HDL cholesterol esters, but positively with their uptake after CETP-mediated transfer to more buoyant lipoprotein fractions; if such a relationship held for the data here, variability in CETP activity might account for variability in determination of these parameters. CETP activity toward endogenous substrates was measured in all animals in terms of the rate of mass transfer between the HDL and LDL pools. This transfer was rapid with little evidence for net mass transfer. Since this exchange reaction had little or no direct effect on determination of the rates of cholesterol ester uptake via the various pathways, it may be taken as an independent measure of CETP activity toward endogenous substrates. This measure of CETP activity (the mean of the unidirectional mass transfer rates between HDL and LDL) correlated inversely with the fraction of HDL cholesterol esters taken up by selective uptake ($r = -0.682$). This indicates that ~ 50% of the variability in selective uptake might be accounted for by variability in CETP activity.

Another possible contributor to the variability in the role of selective uptake is the inconsistent triglyceride levels in rabbits (5, 35); it might be expected that high triglyceride levels would favor the transfer pathway. Triglycerides were measured in two experiments (seven animals), and levels varied 3.9-fold. The fraction of esters taken up by selective uptake was inversely related to triglyceride levels ($r = -0.717$). These limited data suggest a role of varying triglyceride levels in determining variability in selective uptake.

Whatever the proximate cause(s) of the variability seen in the studies here, it may illustrate that the pathways for uptake of HDL cholesterol esters in rabbits are volatile, responding strongly to moderate metabolic changes. Two such effective changes may be CETP activity and triglyceride levels. However, clear conclusions require studies of animals in which these and other candidate factors are directly perturbed.

Although selective uptake played a substantial role in clearance of HDL cholesterol esters, CETP-mediated transfer to more buoyant lipoproteins was the major route for their removal from plasma. This process was mediated at least mostly by the net transport of cholesterol esters to the VLDL fraction, lending support to earlier studies indicating that CETP mediates the exchange of neutral lipids, and that net transfer of cholesterol esters occurs only when they are exchanged for triglycerides (4).

Uptake of HDL particles accounted for only 10% of HDL cholesterol ester clearance. This includes particles both with and without apo E since the plasma decay kinetics of cholesterol ester tracers in the two were not apparently different. This simplification, as well as the simplification of ignoring other types of metabolic heterogeneity (34), was invoked in order to allow better definition of other kinetic parameters. This should not be taken to mean that apo E did not affect the metabolism of HDL particles. For example, the results are not inconsistent with a large contribution of apo E to the clearance of HDL particles. Our data indicate only that cholesterol esters in particles with apo E are not cleared at a substantially greater rate than particles without apo E. This conclusion is not novel to

this study nor to rabbits. Eisenberg et al. (35) concluded that in rats, which lack plasma CETP activity (21), the clearance of esters from HDL particles with apo E is not detectably different from the clearance of esters from particles without apo E.

This study does not directly indicate that the pathway representing the direct removal of cholesterol esters from the HDL pool, which we have called "selective uptake", is necessarily the same selective uptake pathway we have studied in rats and in cell culture systems. Alternative explanations for the data might be proposed. One is that the apparent selective uptake actually represents the uptake of a small subpopulation of rapidly turning over HDL particles that are rich in cholesterol esters but poor in apo A-I; the rapid turnover of a such a small fraction of the HDL apo A-I pool might have been missed. The possibility that particles with apo E represent such a population of particles poor in apo A-I was examined here, and these particles were found not to behave much differently from the general population of HDL particles. No other known class of particles would support this explanation. Another alternative explanation for the apparent selective uptake is that it actually represents a direct cellular uptake of cholesterol esters mediated by CETP, as has been reported (36). Of course, such a process would not be discernible in this study from what we call selective uptake. We have addressed this possibility in the past, and find no evidence that CETP can mediate direct cellular uptake of cholesterol esters (37). Although neither of these alternative explanations is explicitly ruled out, it seems most probable that the selective uptake estimated here is indeed the same selective uptake process that we have previously studied in rats and demonstrated in rabbit fibroblasts.

Identification of an extravascular exchange pool for HDL cholesterol esters independent of HDL apoproteins adds weight to this conclusion, since the result is in keeping with in vitro evidence that such a pool is involved in selective uptake. According to studies in Y-1 mouse adrenal cortical tumor cells and in primary rat adrenal cortical cells, HDL reversibly inserts cholesterol esters into the plasma membrane, from whence they are irreversibly internalized by an undefined mechanism (28) to a cytoplasmic destination (38). There is an almost linear relationship between the content of cholesterol ester tracer in this membrane pool and irreversible selective uptake (28). In these in vivo studies, an analogous correlation of the rate of selective uptake and the size of the extravascular pool of HDL cholesterol esters was not seen. However, the in vitro data suggest that the plasma membrane pool of cholesterol esters would be substantial even if irreversible selective uptake were negligible (a condition that was not achieved experimentally). Consequently, in these rabbit studies any amount of reversibly cell-associated cholesterol esters that was in parallel to selective uptake might be small compared with the whole body pool of reversibly cell-associated tracer, and the size of the exchange pool would not necessarily correlate with the amount of selective uptake in that animal. Thus, these studies support the existence in vivo of a reversibly cell-associated pool of HDL cholesterol esters, and the characteristics of that pool are not inconsistent with those of the pool described in vitro.

While these studies have quantified the routes for clearance of HDL cholesterol esters in rabbits, they have not directly quantified the participation of these routes in reverse cholesterol transport. That would require not only the determination of the rate of plasma clearance by each pathway, but also the rate of hepatic uptake via each pathway. Such studies can currently be done only by examining a very large number of animals in order to apply compartmental analysis to the tissue content of tracers at various times after injection. However, based on other studies, one can suppose that the pathways quantified here must be pertinent to reverse cholesterol transport. It is generally accepted that uptake of VLDL remnant particles is predominantly into the liver (39, 40), and it has also been shown that more than half of LDL particles are taken up by liver in rabbits (20); thus the transfer pathway would be expected to deliver HDL cholesterol esters mostly to the liver. In the case of selective uptake, the pathway occurs predominantly ($\sim$ 90%) in the liver in rats (10). Mackinnon and coworkers have presented evidence for HDL selective uptake by perfused rabbit liver (41), and very recently Wishart and Mackinnon reported selective uptake by cultured rabbit hepatocytes (42); thus it is not unlikely that selective uptake delivers cholesterol esters predominantly to the liver of rabbits. Consequently, this study strongly suggests a role for selective uptake, as well as the transfer pathway, in reverse cholesterol transport in rabbits. This makes it probable that selective uptake plays a role in reverse cholesterol transport in man as well.

Appendix

Data and modeling results for individual rabbits and individual experiments are shown in Tables I–V. The fractional transfer rate $L(i, j)$ is the fraction of tracer or cholesterol ester mass in pool j transferred to pool i per h. The mass transport rate $R(i, j)$ is the flux of cholesterol ester mass (mg cholesterol/dl per h) from pool j to pool i. Compartment numbering is shown in Fig. A1.

Table I, Part 1
Fractional Rate Constants for Compartmental Model of Plasma Cholesterol Esters

Study	Rabbit	L(0,6)	L(16,6)	L(6,16)	L(0,3)	L(13,3)	L(3,13)	L(0,1)	L(21,1)	Total VLDL Outs L(0,1)+L(21,1)	L(0,21)
		(h^{-1})									
Studies Using Cholesteryl Ethers											
1	1	0.016 ± 0.006	0.078 ± 0.098	0.210 ± 0.188	0.018 ± 0.005	0.085 ± 0.059	0.255 ± 0.178	0.144 ± 0.036	0.025 ± 0.014	0.168	0.051 ± 0.020
1	2	0.028 ± 0.006	0.061 ± 0.046	0.202 ± 0.198	0.032 ± 0.005	0.106 ± 0.052	0.258 ± 0.142	0.064 ± 0.039	0.074 ± 0.018	0.137	0.115 ± 0.012
1	3	0.035 ± 0.006	0.088 ± 0.085	0.251 ± 0.264	0.041 ± 0.005	0.114 ± 0.062	0.271 ± 0.147	0.120 ± 0.032	0.038 ± 0.011	0.158	0.092 ± 0.012
Mean of Study 1		0.026 ± 0.009	0.076 ± 0.014	0.221 ± 0.026	0.031 ± 0.012	0.102 ± 0.015	0.261 ± 0.008	0.109 ± 0.041	0.045 ± 0.025	0.154 ± 0.016	0.086 ± 0.032
2	1	0.043 ± 0.005	0.232 ± 0.105	0.588 ± 0.230	0.036 ± 0.005	0.121 ± 0.047	0.239 ± 0.111	0.196 ± 0.027	0.022 ± 0.006	0.218	0.025 ± 0.009
2	2	0.056 ± 0.004	0.193 ± 0.155	0.891 ± 0.734	0.061 ± 0.006	0.420 ± 0.177	0.818 ± 0.287	0.220 ± 0.025	0.018 ± 0.010	0.238	0.000 ± 0.057
2	3	0.069 ± 0.007	0.297 ± 0.170	0.975 ± 0.591	0.054 ± 0.005	0.088 ± 0.101	0.482 ± 0.450	0.142 ± 0.021	0.020 ± 0.007	0.162	0.013 ± 0.014
Mean of Study 2		0.056 ± 0.013	0.241 ± 0.053	0.818 ± 0.204	0.050 ± 0.013	0.209 ± 0.183	0.513 ± 0.291	0.186 ± 0.040	0.020 ± 0.002	0.206 ± 0.039	0.013 ± 0.012
3	1	0.031 ± 0.006	0.113 ± 0.064	0.227 ± 0.148	0.046 ± 0.009	0.102 ± 0.023	0.101 ± 0.046	0.400 ± 0.055	0.025 ± 0.005	0.424	0.022 ± 0.007
3	2	0.035 ± 0.006	0.123 ± 0.058	0.232 ± 0.134	0.047 ± 0.005	0.097 ± 0.039	0.206 ± 0.095	0.319 ± 0.048	0.014 ± 0.005	0.333	0.029 ± 0.013
3	3	0.032 ± 0.005	0.088 ± 0.061	0.250 ± 0.215	0.037 ± 0.004	0.101 ± 0.051	0.291 ± 0.142	0.195 ± 0.037	0.015 ± 0.007	0.209	0.003 ± 0.016
3	4	0.037 ± 0.008	0.088 ± 0.052	0.182 ± 0.148	0.044 ± 0.005	0.175 ± 0.069	0.312 ± 0.113	0.163 ± 0.042	0.012 ± 0.009	0.175	0.000 ± 0.029
Mean of Study 3		0.034 ± 0.003	0.103 ± 0.018	0.223 ± 0.029	0.043 ± 0.005	0.119 ± 0.037	0.227 ± 0.096	0.269 ± 0.110	0.016 ± 0.006	0.286 ± 0.115	0.013 ± 0.014
Mean of Rabbits Receiving Cholesteryl Ethers		0.038 ± 0.015	0.136 ± 0.078	0.401 ± 0.304	0.042 ± 0.012	0.141 ± 0.101	0.323 ± 0.198	0.196 ± 0.099	0.026 ± 0.018	0.222 ± 0.091	0.035 ± 0.040
Studies Using Cholesterol Esters											
4	1	0.043 ± 0.006	0.181 ± 0.107	0.473 ± 0.233	0.093 ± 0.010	0.204 ± 0.066	0.238 ± 0.068	0.311 ± 0.054	0.004 ± 0.002	0.316	0.000 ± 0.027
4	2	0.036 ± 0.007	0.114 ± 0.052	0.221 ± 0.072	0.083 ± 0.008	0.080 ± 0.038	0.184 ± 0.087	0.392 ± 0.120	0.001 ± 0.009	0.393	0.000 ± 0.923
4	3	0.033 ± 0.006	0.300 ± 0.173	0.697 ± 0.349	0.073 ± 0.009	0.136 ± 0.046	0.198 ± 0.068	0.435 ± 0.173	0.028 ± 0.008	0.463	0.043 ± 0.015
Mean of Rabbits Receiving Cholesterol Esters		0.038 ± 0.005	0.199 ± 0.094	0.464 ± 0.238	0.083 ± 0.010	0.140 ± 0.062	0.207 ± 0.028	0.380 ± 0.063	0.011 ± 0.015	0.391 ± 0.074	0.014 ± 0.025
All Studies											
Mean of all Rabbits		0.038 ± 0.013	0.151 ± 0.083	0.415 ± 0.282	0.051 ± 0.021	0.141 ± 0.091	0.296 ± 0.179	0.238 ± 0.120	0.023 ± 0.018	0.261 ± 0.112	0.030 ± 0.037

Table I, Part 2
Fractional Rate Constants for Compartmental Model of Plasma Cholesterol Esters

Study	Rabbit	L(1,6)	L(6,1)	L(3,6)	L(6,3)	L(3,1)	L(1,3)	L(10,6)	L(7,6)	L(6,7)
		(h^{-1})								
Studies Using Cholesteryl Ethers										
1	1	0.338 ± 0.040	0.127 ± 0.017	0.411 ± 0.077	0.135 ± 0.023	0.133 ± 0.020	0.000 ± 0.017	0.004 ± 0.047	0.030 ± 0.154	0.302
1	2	0.093 ± 0.007	0.221 ± 0.018	0.254 ± 0.027	0.103 ± 0.012	0.190 ± 0.018	0.000 ± 0.006	0.078 ± 0.020	0.242 ± 0.110	0.389 ± 0.166
1	3	0.085 ± 0.009	0.162 ± 0.016	0.220 ± 0.032	0.081 ± 0.013	0.152 ± 0.015	0.000 ± 0.007	0.110 ± 0.023	0.257 ± 0.125	0.268 ± 0.122
Mean of Study 1		0.172 ± 0.144	0.170 ± 0.047	0.295 ± 0.102	0.106 ± 0.027	0.158 ± 0.029	0.000 ± 0.000	0.064 ± 0.054	0.177 ± 0.127	0.320 ± 0.063
2	1	0.200 ± 0.019	0.103 ± 0.010	0.288 ± 0.056	0.052 ± 0.006	0.111 ± 0.011	0.000 ± 0.007	0.246 ± 0.048	0.000 ± 0.089	0.302
2	2	0.295 ± 0.021	0.092 ± 0.009	0.334 ± 0.079	0.083 ± 0.012	0.129 ± 0.014	0.000 ± 0.010	0.252 ± 0.051	0.000 ± 0.098	0.302
2	3	0.380 ± 0.036	0.097 ± 0.011	0.225 ± 0.079	0.046 ± 0.008	0.066 ± 0.012	0.000 ± 0.018	0.177 ± 0.064	0.000 ± 0.105	0.302
Mean of Study 2		0.292 ± 0.090	0.097 ± 0.005	0.283 ± 0.055	0.060 ± 0.020	0.102 ± 0.032	0.000 ± 0.000	0.225 ± 0.042	0.000 ± 0.000	0.302 ± 0.000
3	1	0.329 ± 0.031	0.190 ± 0.026	0.415 ± 0.054	0.154 ± 0.021	0.198 ± 0.025	0.000 ± 0.016	0.000 ± 0.052	0.145 ± 0.132	0.405 ± 0.328
3	2	0.223 ± 0.015	0.208 ± 0.019	0.418 ± 0.051	0.151 ± 0.019	0.196 ± 0.022	0.000 ± 0.009	0.031 ± 0.029	0.166 ± 0.106	0.328 ± 0.201
3	3	0.510 ± 0.032	0.170 ± 0.014	0.520 ± 0.064	0.130 ± 0.015	0.218 ± 0.019	0.000 ± 0.014	0.000 ± 0.050	0.132 ± 0.101	0.351 ± 0.251
3	4	0.778 ± 0.065	0.160 ± 0.017	0.490 ± 0.085	0.202 ± 0.031	0.173 ± 0.020	0.000 ± 0.035	0.000 ± 0.097	0.320 ± 0.118	0.206 ± 0.075
Mean of Study 3		0.460 ± 0.243	0.182 ± 0.021	0.461 ± 0.053	0.159 ± 0.031	0.196 ± 0.019	0.000 ± 0.000	0.008 ± 0.016	0.191 ± 0.087	0.322 ± 0.084
Mean of Rabbits Receiving Cholesteryl Ethers		0.323 ± 0.206	0.153 ± 0.046	0.358 ± 0.109	0.114 ± 0.049	0.157 ± 0.047	0.000 ± 0.000	0.090 ± 0.102	0.129 ± 0.119	0.316 ± 0.058
Studies Using Cholesterol Esters										
4	1	0.059 ± 0.019	0.228 ± 0.049	0.737 ± 0.147	0.262 ± 0.060	0.052 ± 0.039	0.033 ± 0.009	0.072 ± 0.045	0.000 ± 0.144	0.302
4	2	0.374 ± 0.064	0.495 ± 0.088	0.330 ± 0.092	0.249 ± 0.065	0.112 ± 0.048	0.000 ± 0.044	0.139 ± 0.075	0.041 ± 0.100	0.302
4	3	0.246 ± 0.068	0.603 ± 0.149	0.759 ± 0.216	0.385 ± 0.109	0.158 ± 0.111	0.036 ± 0.035	0.043 ± 0.067	0.005 ± 0.129	0.302
Mean of Rabbits Receiving Cholesterol Esters		0.226 ± 0.159	0.442 ± 0.193	0.608 ± 0.241	0.299 ± 0.075	0.107 ± 0.053	0.023 ± 0.020	0.085 ± 0.050	0.015 ± 0.022	0.302 ± 0.000
All Studies										
Mean of all Rabbits		0.301 ± 0.194	0.220 ± 0.155	0.416 ± 0.175	0.156 ± 0.097	0.145 ± 0.051	0.005 ± 0.013	0.089 ± 0.091	0.103 ± 0.115	0.312 ± 0.050

Table II
Cholesterol Ester Masses and Production Rates

Study	Rabbit	M(1)	M(3)	M(6)	M(13)	M(16)	M(7)	U(1)	U(3)	U(6)
		mg cholesterol/dl						mg cholesterol/dl/h		
Studies Using Cholesteryl Ethers										
2	1	4.8	23.7	7.8	11.9 ± 2.7	3.1 ± 1.1	0.0 ± 2.3	0.51 ± 0.13	-0.69 ± 0.25	4.32 ± 0.32
2	2	4.6	17.3	7.1	8.9 ± 1.6	1.5 ± 0.7	0.0 ± 2.3	0.02 ± 0.16	-0.48 ± 0.31	4.81 ± 0.36
2	3	10.7	24.4	12.9	4.4 ± 2.1	3.9 ± 1.6	0.0 ± 4.5	-1.43 ± 0.34	-1.18 ± 0.61	8.83 ± 0.74
Mean of Study 2		6.7 ± 3.5	21.8 ± 3.9	9.3 ± 3.2	8.4 ± 3.8	2.9 ± 1.2	0.0 ± 0.0	-0.30 ± 1.01	-0.78 ± 0.36	5.99 ± 2.47
3	1	9.2	14.1	13.6	14.2 ± 4.0	6.8 ± 2.1	4.9 ± 3.6	2.99 ± 0.32	-4.65 ± 0.48	6.62 ± 0.58
3	2	5.7	45.9	16.3	21.6 ± 5.1	8.6 ± 2.8	8.3 ± 4.8	0.57 ± 0.33	1.15 ± 0.33	3.42 ± 0.37
3	3	18.7	29.9	14.2	10.4 ± 2.5	5.0 ± 1.9	5.3 ± 5.4	3.94 ± 0.51	-6.47 ± 0.57	8.01 ± 0.58
3	4	16.4	43.9	14.1	24.6 ± 5.1	6.8 ± 3.1	22.0 ± 8.3	-2.63 ± 1.26	1.05 ± 0.61	6.90 ± 0.70
Mean of Study 3		12.5 ± 6.1	33.5 ± 14.7	14.6 ± 1.2	17.7 ± 6.5	6.8 ± 1.5	10.1 ± 8.0	1.22 ± 2.93	-2.23 ± 3.92	6.24 ± 1.97
Mean of Rabbits Receiving Cholesteryl Ethers		10.0 ± 5.7	28.5 ± 12.3	12.3 ± 3.5	13.7 ± 7.1	5.1 ± 2.5	5.8 ± 7.8	0.57 ± 2.30	-1.61 ± 2.88	6.13 ± 2.00
Studies Using Cholesterol Esters										
4	1	4.3	65.7	27.7	56.1 ± 9.5	10.6 ± 4.5	0.0 ± 13.2	-1.22 ± 0.25	4.81 ± 0.96	7.03 ± 1.19
4	2	5.5	34.9	16.5	15.2 ± 4.0	8.5 ± 3.0	2.2 ± 5.5	-0.67 ± 1.03	5.52 ± 0.64	3.10 ± 1.18
4	3	4.4	35.3	15.7	24.2 ± 4.8	6.8 ± 2.3	0.3 ± 6.7	0.24 ± 0.59	4.83 ± 0.81	0.74 ± 1.00
Mean of Rabbits Receiving Cholesterol Esters		4.7 ± 0.7	45.3 ± 17.7	20.0 ± 6.7	31.8 ± 21.5	8.6 ± 1.9	0.8 ± 1.2	-0.55 ± 0.73	5.05 ± 0.40	3.62 ± 3.18
All Studies										
Mean of all Rabbits		8.4 ± 5.3	33.5 ± 15.4	14.6 ± 5.6	19.2 ± 14.6	6.2 ± 2.8	4.3 ± 6.9	0.23 ± 1.98	0.39 ± 3.99	5.38 ± 2.52

Table III, Part 1
Cholesterol Ester Transport Rates

Study	Rabbit	R(0,6)	R(16,6) R(6,16)	R(0,3)	R(13,3) R(3,13)	R(0,1)	R(21,1) R(0,21)	Total VLDL Outs R(0,1)+R(21,1)
		(mg cholesterol/dl/h)						
Studies Using Cholesteryl Ethers								
2	1	0.33 ± 0.04	1.81 ± 0.82	0.85 ± 0.13	2.86 ± 1.13	0.94 ± 0.13	0.10 ± 0.03	1.04 ± 0.13
2	2	0.40 ± 0.03	1.37 ± 1.10	1.06 ± 0.10	7.27 ± 3.06	1.01 ± 0.12	0.08 ± 0.05	1.09 ± 0.13
2	3	0.89 ± 0.09	3.84 ± 2.19	1.31 ± 0.12	2.14 ± 2.45	1.52 ± 0.22	0.21 ± 0.07	1.73 ± 0.25
Mean of Study 2		0.54 ± 0.31	2.34 ± 1.32	1.08 ± 0.23	4.09 ± 2.78	1.16 ± 0.32	0.13 ± 0.07	1.29 ± 0.38
3	1	0.42 ± 0.08	1.53 ± 0.88	0.64 ± 0.13	1.43 ± 0.32	3.68 ± 0.51	0.23 ± 0,05	3.91 ± 0.52
3	2	0.57 ± 0.10	2.00 ± 0.95	2.17 ± 0.24	4.43 ± 1.81	1.82 ± 0.27	0.08 ± 0.03	1.90 ± 0.28
3	3	0.46 ± 0.07	1.24 ± 0.87	1.10 ± 0.12	3.03 ± 1.52	3.64 ± 0.68	0.27 ± 0.13	3.91 ± 0.71
3	4	0.52 ± 0.11	1.24 ± 0.74	1.92 ± 0.23	7.66 ± 3.05	2.68 ± 0.69	0.20 ± 0.14	2.87 ± 0.72
Mean of Study 3		0.49 ± 0.07	1.51 ± 0.36	1.46 ± 0.71	4.14 ± 2.65	2.95 ± 0.89	0.20 ± 0.08	3.15 ± 0.96
Mean of Rabbits Receiving Cholesteryl Ethers		0.51 ± 0.19	1.86 ± 0.92	1.29 ± 0.56	4.12 ± 2.47	2.18 ± 1.16	0.17 ± 0.08	2.35 ± 1.22
Studies Using Cholesterol Esters								
4	1	1.20 ± 0.17	5.02 ± 2.95	6.08 ± 0.65	13.38 ± 4.36	1.34 ± 0.23	0.02 ± 0.01	1.36 ± 0.24
4	2	0.60 ± 0.11	1.89 ± 0.86	2.89 ± 0.28	2.80 ± 1.31	2.16 ± 0.66	0.01 ± 0.05	2.16 ± 0.67
4	3	0.52 ± 0.09	4.71 ± 2.71	2.58 ± 0.30	4.80 ± 1.62	1.91 ± 0.76	0.12 ± 0.04	2.03 ± 0.76
Mean of Rabbits Receiving Cholesterol Esters		0.77 ± 0.37	3.87 ± 1.73	3.85 ± 1.94	6.99 ± 5.62	1.80 ± 0.42	0.05 ± 0.06	1.85 ± 0.43
All Studies								
Mean of all Rabbits		0.59 ± 0.26	2.47 ± 1.47	2.06 ± 1.60	4.98 ± 3.60	2.07 ± 0.99	0.13 ± 0.09	2.20 ± 1.05

Table III, Part 2
Cholesterol Ester Transport Rates

Study	Rabbit	R(1,6)	R(6,1)	R(3,6)	R(6,3)	R(3,1)	R(1,3)	R(10,6)	R(7,6) R(6,7)
		(mg cholesterol/dl/h)							
Studies Using Cholesteryl Ethers									
2	1	1.56 ± 0.15	0.49 ± 0.05	2.25 ± 0.44	1.24 ± 0.15	0.53 ± 0.05	0.00 ± 0.16	1.92 ± 0.37	0.00 ± 0.69
2	2	2.10 ± 0.15	0.42 ± 0.04	2.37 ± 0.56	1.43 ± 0.20	0.59 ± 0.06	0.00 ± 0.17	1.79 ± 0.36	0.00 ± 0.69
2	3	4.91 ± 0.47	1.04 ± 0.11	2.91 ± 1.02	1.12 ± 0.19	0.71 ± 0.13	0.00 ± 0.43	2.28 ± 0.82	0.00 ± 1.36
Mean of Study 2		2.85 ± 1.80	0.65 ± 0.34	2.51 ± 0.35	1.26 ± 0.16	0.61 ± 0.09	0.00 ± 0.00	1.99 ± 0.25	0.00 ± 0.00
3	1	4.47 ± 0.42	1.74 ± 0.24	5.64 ± 0.74	2.17 ± 0.29	1.82 ± 0.23	0.00 ± 0.22	0.00 ± 0.71	1.97 ± 1.80
3	2	3.63 ± 0.25	1.18 ± 0.11	6.81 ± 0.83	6.91 ± 0.86	1.12 ± 0.12	0.00 ± 0.41	0.51 ± 0.48	2.70 ± 1.73
3	3	7.24 ± 0.45	3.19 ± 0.27	7.38 ± 0.91	3.89 ± 0.44	4.08 ± 0.36	0.00 ± 0.42	0.00 ± 0.72	1.88 ± 1.44
3	4	10.97 ± 0.92	2.63 ± 0.27	6.91 ± 1.19	8.87 ± 1.36	2.83 ± 0.32	0.00 ± 1.54	0.00 ± 1.37	4.52 ± 1.66
Mean of Study 3		6.58 ± 3.31	2.19 ± 0.89	6.69 ± 0.74	5.46 ± 3.00	2.46 ± 1.29	0.00 ± 0.00	0.13 ± 0.25	2.77 ± 1.22
Mean of Rabbits Receiving Cholesteryl Ethers		4.98 ± 3.24	1.53 ± 1.05	4.90 ± 2.30	3.66 ± 3.09	1.67 ± 1.35	0.00 ± 0.00	0.93 ± 1.02	1.58 ± 1.71
Studies Using Cholesterol Esters									
4	1	1.63 ± 0.53	0.98 ± 0.21	20.41 ± 4.08	17.22 ± 3.97	0.22 ± 0.17	2.14 ± 0.58	1.99 ± 1.25	0.00 ± 3.98
4	2	6.17 ± 1.06	2.72 ± 0.48	5.44 ± 1.52	8.69 ± 2.28	0.62 ± 0.26	0.00 ± 1.53	2.30 ± 1.24	0.67 ± 1.65
4	3	3.86 ± 1.06	2.65 ± 0.65	11.91 ± 3.39	13.58 ± 3.84	0.69 ± 0.49	1.27 ± 1.22	0.67 ± 1.06	0.08 ± 2.02
Mean of Rabbits Receiving Cholesterol Esters		3.89 ± 2.27	2.12 ± 0.99	12.59 ± 7.51	13.16 ± 4.28	0.51 ± 0.25	1.14 ± 1.08	1.65 ± 0.86	0.25 ± 0.36
All Studies									
Mean of all Rabbits		4.65 ± 2.90	1.70 ± 1.02	7.20 ± 5.46	6.51 ± 5.61	1.32 ± 1.24	0.34 ± 0.75	1.14 ± 0.99	1.18 ± 1.55

Table IV
Net Interlipoprotein Cholesterol Ester Transport Rates

Study	Rabbit	HDL to VLDL R(1,6) - R(6,1)	HDL to LDL R(3,6) - R(6,3)	VLDL to LDL R(3,1) - R(1,3)
		(mg cholesterol/dl/h)		
Studies Using Cholesteryl Ethers				
2	1	1.07 ± 0.15	1.01 ± 0.36	0.53 ± 0.17
2	2	1.67 ± 0.15	0.95 ± 0.42	0.59 ± 0.18
2	3	3.87 ± 0.47	1.79 ± 0.93	0.71 ± 0.41
Mean of Study 2		2.20 ± 1.47	1.25 ± 0.47	0.61 ± 0.09
3	1	2.73 ± 0.47	3.47 ± 0.57	1.82 ± 0.29
3	2	2.45 ± 0.28	-0.10 ± 0.50	1.12 ± 0.40
3	3	4.05 ± 0.51	3.49 ± 0.70	4.08 ± 0.51
3	4	8.34 ± 1.00	-1.96 ± 1.24	2.83 ± 1.49
Mean of Study 3		4.39 ± 2.72	1.23 ± 2.71	2.46 ± 1.29
Mean of Rabbits Receiving Cholesteryl Ethers		3.45 ± 2.41	1.24 ± 1.94	1.67 ± 1.35
Studies Using Cholesterol Esters				
4	1	0.65 ± 0.57	3.19 ± 1.16	-1.92 ± 0.58
4	2	3.45 ± 1.17	-3.25 ± 1.52	0.62 ± 1.54
4	3	1.21 ± 1.23	-1.67 ± 1.42	-0.58 ± 1.32
Mean of Rabbits Receiving Cholesterol Esters		1.77 ± 1.48	-0.57 ± 3.36	-0.63 ± 1.27
All Studies				
Mean of all Rabbits		2.95 ± 2.24	0.69 ± 2.40	0.98 ± 1.67

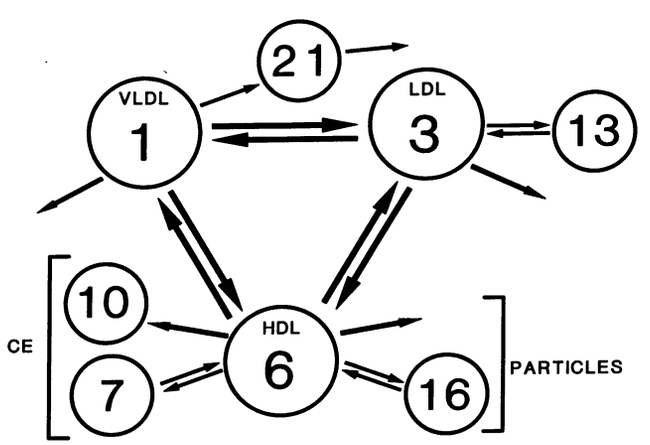

Figure A1.

Table V, Part 1
Cholesterol Ester Transport Rates Normalized to HDL Production Rate

Study	Rabbit	R(0,6)	R(16,6) R(6,16)	R(0,3)	R(13,3) R(3,13)	R(0,1)	R(21,1) R(0,21)	Total VLDL Outs R(0,1)+R(21,1)
		(fraction of HDL production)						
Studies Using Cholesteryl Ethers								
1	1	0.077 ± 0.031	0.365 ± 0.459	0.287 ± 0.073	1.332 ± 0.913	0.526 ± 0.181	0.091 ± 0.042	0.618 ± 0.182
1	2	0.141 ± 0.031	0.308 ± 0.236	0.346 ± 0.049	1.132 ± 0.546	0.054 ± 0.035	0.063 ± 0.013	0.117 ± 0.038
1	3	0.135 ± 0.025	0.343 ± 0.333	0.326 ± 0.042	0.899 ± 0.485	0.084 ± 0.026	0.026 ± 0.007	0.111 ± 0.028
Mean of Study 1		0.118 ± 0.036	0.339 ± 0.029	0.320 ± 0.030	1.121 ± 0.217	0.222 ± 0.264	0.060 ± 0.033	0.282 ± 0.291
2	1	0.081 ± 0.009	0.440 ± 0.190	0.262 ± 0.052	0.880 ± 0.356	0.172 ± 0.032	0.019 ± 0.005	0.191 ± 0.033
2	2	0.088 ± 0.007	0.302 ± 0.230	0.279 ± 0.044	1.908 ± 0.857	0.221 ± 0.034	0.018 ± 0.009	0.239 ± 0.034
2	3	0.116 ± 0.012	0.496 ± 0.270	0.273 ± 0.054	0.445 ± 0.536	0.277 ± 0.052	0.039 ± 0.012	0.316 ± 0.055
Mean of Study 2		0.095 ± 0.018	0.413 ± 0.100	0.271 ± 0.009	1.078 ± 0.751	0.223 ± 0.052	0.025 ± 0.012	0.249 ± 0.063
3	1	0.097 ± 0.020	0.357 ± 0.207	0.359 ± 0.056	0.800 ± 0.154	0.513 ± 0.098	0.032 ± 0.006	0.545 ± 0.100
3	2	0.124 ± 0.024	0.437 ± 0.210	0.406 ± 0.048	0.830 ± 0.329	0.344 ± 0.067	0.015 ± 0.005	0.359 ± 0.068
3	3	0.088 ± 0.014	0.239 ± 0.168	0.425 ± 0.051	1.172 ± 0.581	0.453 ± 0.107	0.034 ± 0.014	0.487 ± 0.107
3	4	0.083 ± 0.019	0.201 ± 0.119	0.306 ± 0.044	1.220 ± 0.490	0.568 ± 0.189	0.042 ± 0.026	0.610 ± 0.188
Mean of Study 3		0.098 ± 0.018	0.308 ± 0.108	0.374 ± 0.053	1.005 ± 0.221	0.470 ± 0.096	0.031 ± 0.011	0.500 ± 0.107
Mean of Rabbits Receiving Cholesteryl Ethers		0.103 ± 0.024	0.349 ± 0.092	0.327 ± 0.056	1.062 ± 0.393	0.321 ± 0.188	0.038 ± 0.023	0.359 ± 0.196
Studies Using Cholesterol Esters								
4	1	0.121 ± 0.018	0.506 ± 0.287	0.499 ± 0.074	1.097 ± 0.379	0.178 ± 0.044	0.002 ± 0.001	0.181 ± 0.045
4	2	0.088 ± 0.017	0.275 ± 0.130	0.223 ± 0.035	0.216 ± 0.111	0.353 ± 0.138	0.001 ± 0.008	0.354 ± 0.139
4	3	0.108 ± 0.018	0.970 ± 0.511	0.382 ± 0.057	0.710 ± 0.253	0.350 ± 0.164	0.022 ± 0.006	0.372 ± 0.166
Mean of Rabbits Receiving Cholesterol Esters		0.105 ± 0.017	0.584 ± 0.354	0.368 ± 0.138	0.674 ± 0.442	0.294 ± 0.100	0.009 ± 0.012	0.302 ± 0.106
All Studies								
Mean of all Rabbits		0.104 ± 0.022	0.403 ± 0.194	0.336 ± 0.077	0.972 ± 0.421	0.315 ± 0.169	0.031 ± 0.025	0.346 ± 0.177

Table V, Part 2
Cholesterol Ester Transport Rates Normalized to HDL Production Rate

Study	Rabbit	R(1,6)	R(6,1)	R(3,6)	R(6,3)	R(3,1)	R(1,3)	R(10,6)	R(7,6) R(6,7)
		(fraction of HDL production)							
Studies Using Cholesteryl Ethers									
1	1	1.574 ± 0.248	0.467 ± 0.079	1.915 ± 0.410	2.116 ± 0.419	0.488 ± 0.089	0.000 ± 0.244	0.019 ± 0.221	0.141 ± 0.722
1	2	0.469 ± 0.053	0.189 ± 0.017	1.285 ± 0.153	1.101 ± 0.152	0.162 ± 0.016	0.000 ± 0.042	0.395 ± 0.082	1.223 ± 0.530
1	3	0.330 ± 0.045	0.113 ± 0.011	0.855 ± 0.125	0.635 ± 0.114	0.106 ± 0.011	0.000 ± 0.032	0.428 ± 0.068	0.999 ± 0.460
Mean of Study 1		0.791 ± 0.681	0.257 ± 0.186	1.352 ± 0.533	1.284 ± 0.757	0.252 ± 0.206	0.000 ± 0.000	0.281 ± 0.227	0.788 ± 0.571
2	1	0.379 ± 0.044	0.090 ± 0.008	0.547 ± 0.104	0.383 ± 0.062	0.098 ± 0.010	0.000 ± 0.025	0.466 ± 0.070	0.000 ± 0.169
2	2	0.462 ± 0.042	0.093 ± 0.009	0.523 ± 0.119	0.375 ± 0.077	0.130 ± 0.013	0.000 ± 0.029	0.394 ± 0.065	0.000 ± 0.152
2	3	0.635 ± 0.076	0.189 ± 0.019	0.376 ± 0.129	0.233 ± 0.060	0.130 ± 0.022	0.000 ± 0.049	0.295 ± 0.093	0.000 ± 0.176
Mean of Study 2		0.492 ± 0.131	0.124 ± 0.057	0.482 ± 0.092	0.330 ± 0.084	0.119 ± 0.019	0.000 ± 0.000	0.385 ± 0.086	0.000 ± 0.000
3	1	1.042 ± 0.125	0.243 ± 0.036	1.313 ± 0.183	1.208 ± 0.195	0.254 ± 0.036	0.000 ± 0.114	0.000 ± 0.125	0.459 ± 0.402
3	2	0.794 ± 0.079	0.224 ± 0.024	1.489 ± 0.194	1.294 ± 0.187	0.211 ± 0.025	0.000 ± 0.071	0.111 ± 0.101	0.591 ± 0.366
3	3	1.392 ± 0.122	0.397 ± 0.048	1.419 ± 0.189	1.502 ± 0.192	0.508 ± 0.065	0.000 ± 0.153	0.000 ± 0.134	0.360 ± 0.271
3	4	1.771 ± 0.214	0.558 ± 0.083	1.116 ± 0.215	1.412 ± 0.246	0.602 ± 0.104	0.000 ± 0.244	0.000 ± 0.214	0.729 ± 0.249
Mean of Study 3		1.249 ± 0.425	0.355 ± 0.156	1.334 ± 0.162	1.354 ± 0.129	0.394 ± 0.191	0.000 ± 0.000	0.028 ± 0.055	0.535 ± 0.160
Mean of Rabbits Receiving Cholesteryl Ethers		0.885 ± 0.530	0.256 ± 0.163	1.084 ± 0.496	1.026 ± 0.605	0.269 ± 0.190	0.000 ± 0.000	0.211 ± 0.202	0.450 ± 0.436
Studies Using Cholesterol Esters									
4	1	0.164 ± 0.056	0.130 ± 0.024	2.057 ± 0.452	1.412 ± 0.391	0.030 ± 0.022	0.176 ± 0.048	0.200 ± 0.113	0.000 ± 0.401
4	2	0.901 ± 0.189	0.445 ± 0.077	0.794 ± 0.240	0.672 ± 0.228	0.101 ± 0.044	0.000 ± 0.117	0.335 ± 0.166	0.098 ± 0.248
4	3	0.796 ± 0.240	0.485 ± 0.109	2.452 ± 0.748	2.009 ± 0.686	0.127 ± 0.086	0.189 ± 0.178	0.138 ± 0.208	0.017 ± 0.415
Mean of Rabbits Receiving Cholesterol Esters		0.620 ± 0.398	0.354 ± 0.194	1.768 ± 0.866	1.364 ± 0.670	0.086 ± 0.050	0.121 ± 0.105	0.224 ± 0.101	0.038 ± 0.052
All Studies									
Mean of all Rabbits		0.824 ± 0.500	0.279 ± 0.168	1.242 ± 0.632	1.104 ± 0.609	0.227 ± 0.184	0.028 ± 0.068	0.214 ± 0.180	0.355 ± 0.419

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