Diet modification alters plasma HDL cholesterol concentrations but not the transport of HDL cholesteryl esters to the liver in the hamster

Laura A. Woollett, Denise M. Kearney, and David K. Spady[1]

Department of Internal Medicine, The University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, TX 75235-8887

Abstract These studies were undertaken to investigate the mechanism whereby diet modification alters the plasma concentration of high density lipoprotein (HDL) cholesteryl ester and apoA-I and to determine whether diet-induced alterations in circulating HDL levels are associated with changes in the rate of reverse cholesterol transport. Rates of HDL cholesteryl ester and apoA-I transport were measured in hamsters fed a control low-cholesterol, low-fat diet or the same diet supplemented with soluble fiber (psyllium) or with cholesterol and triglyceride (Western-type diet). The Western-type diet increased the plasma concentration of HDL cholesteryl ester by 46% compared to the control diet and by 86% compared to the psyllium-supplemented diet; nevertheless, the absolute rates of HDL cholesteryl ester transport to the liver were identical in the three groups. Diet-induced alterations in circulating HDL cholesteryl ester levels were due to changes in the rate of HDL cholesteryl ester entry into HDL (whole body HDL cholesteryl ester transport) and not to regulation of HDL cholesteryl ester clearance mechanisms. The Western-type diet increased the plasma concentration of HDL apoA-I by 25% compared to the control diet and by 45% relative to the psyllium-supplemented diet. Diet-induced alterations in plasma HDL apoA-I concentrations were also due entirely to changes in the rate of apoA-I entry into HDL (whole body HDL apoA-I transport). These studies demonstrate that the absolute flux of HDL cholesteryl ester to the liver, which reflects the rate of reverse cholesterol transport, remains constant under conditions in which plasma HDL cholesteryl ester concentrations are altered over a nearly 2-fold range by diet modification.—**Woollett, L. A., D. M. Kearney, and D. K. Spady.** Diet modification alters plasma HDL cholesterol concentrations but not the transport of HDL cholesteryl esters to the liver in the hamster. *J. Lipid Res.* 1997. **38:** 2289–2302.

Supplementary key words HDL • reverse cholesterol transport • cholesteryl ester • liver • cholesterol synthesis • lipoproteins

Epidemiological data and clinical trials have shown that the risk of developing coronary heart disease is directly related to the plasma concentration of low density lipoprotein (LDL) cholesterol and inversely related to the plasma concentration of high density lipoprotein (HDL) cholesterol (1, 2). A number of dietary factors have been shown to alter the amount of cholesterol carried in plasma LDL and HDL (3–5). In the hamster (6), as in humans (7, 8), those diets most effective in lowering circulating LDL levels generally lower HDL concentrations as well. For example, plasma HDL cholesterol concentrations are increased by dietary cholesterol and triglycerides and are reduced by soluble fibers such as psyllium (6–8). The mechanisms responsible for these effects and the implications with respect to reverse cholesterol transport and atherogenesis are not fully understood. Because the effect of diet modification on cardiovascular mortality has never been studied in a randomized controlled trial, and because diet modification tends to lower the plasma concentration of HDL as well as LDL, there is some concern that the beneficial effects of a lower level of atherogenic LDL particles may be partially offset by the accompanying fall in protective HDL particles.

The protective role of HDL is usually attributed to its ability to transport excess cholesterol from peripheral tissues back to the liver. Although some cholesterol is converted to steroid hormones or lost from the body when cells are sloughed from the skin or gastrointestinal tract, the bulk of cholesterol that is acquired by extrahepatic tissues (via de novo synthesis or lipoprotein

Abbreviations: LDL, low density lipoprotein(s); HDL, high density lipoprotein(s); LCAT, lecithin:cholesterol acyltransferase; CETP, cholesteryl ester transfer protein; apoA-I, apolipoprotein A-I; SR-BI, scavenger receptor type BI; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

[1]To whom correspondence should be addressed.

uptake) must be returned to the liver for excretion in a process that has been termed reverse (9, 10) or centripetal (11) cholesterol transport. Reverse cholesterol transport is initiated in extrahepatic tissues by the transfer of unesterified cholesterol from cell membranes to nascent HDL (12, 13). A portion of this cholesterol is esterified by lecithin:cholesterol acyltransferase (LCAT) and partitions into the hydrophobic core of the HDL particle (12). The cholesteryl esters of HDL are returned to the liver through several pathways. In many species, cholesteryl ester transfer protein (CETP) mediates the transfer of HDL cholesteryl esters to triglyceride-rich lipoproteins, which are ultimately cleared by the liver via the LDL receptor and LDL receptor-related protein (14). HDL cholesteryl esters are also delivered to the liver via a nonendocytic process in which cholesteryl ester is selectively taken up by the liver resulting in an HDL particle of reduced size and cholesteryl ester content (15, 16). Finally, a portion of HDL is taken up by the liver as intact HDL particles (17).

In recent studies we described the kinetic characteristics of HDL cholesteryl ester and apoA-I transport in control hamsters (18). These studies showed that the majority of HDL cholesteryl ester (~75%) is cleared from plasma by a saturable transport process in the liver and that the transport of HDL cholesteryl ester to the liver closely approximates the rate of cholesterol acquisition by the extrahepatic tissues (via lipoprotein uptake and de novo synthesis). It was further shown that the plasma concentration of HDL cholesteryl ester necessary to achieve half-maximal uptake rates in the liver was 14 mg/dl, well below normal HDL cholesteryl ester concentrations in the hamster. As a consequence, HDL cholesteryl ester uptake by the liver is largely saturated at normal HDL concentrations and it can be predicted that alterations in circulating HDL levels (due to dietary factors or pharmacological agents) will not be accompanied by a change in the absolute flux of HDL cholesteryl ester to the liver unless transporter activity is regulated or the plasma HDL concentration is drastically reduced.

The present studies were undertaken to determine whether diet-induced alterations in plasma HDL concentrations are associated with changes in the rate of reverse cholesterol transport in the hamster. The hamster is an attractive model for the study of HDL transport in that HDL levels in this species respond to dietary factors in a manner similar to humans (3, 4, 19). More importantly, the kinetic constants for HDL apoA-I and cholesteryl ester transport have been determined in this species so that it is possible to identify the mechanism whereby a dietary or pharmacological intervention alters plasma HDL concentrations (18).

METHODS

Animals and diets

Male Golden Syrian hamsters (Sasco, Inc., Omaha, NE) were housed in colony cages and subjected to alternating 12-h periods of light and darkness for at least 3 weeks prior to introduction of the experimental diets. During this time, animals were maintained on a standard low-cholesterol (0.23 mg/g), low-fat (45 mg/g) diet (Teklab Premier Laboratory Diets, Madison, WI). Experimental diets were prepared by supplementing the control diet with dietary factors previously found to significantly alter plasma HDL concentrations in the hamster (6). Plasma HDL cholesterol concentrations were raised using a Western-type diet. This diet consisted of the control diet enriched with triglyceride (5% by wt safflower oil and 10% by wt coconut oil) and cholesterol (0.1% by wt) to provide ~36% of total calories as triglyceride and ~217 mg cholesterol per 1000 kcal of diet. Plasma HDL cholesterol levels were lowered by feeding the control diet supplemented with psyllium (5% by wt). These diets were fed ad lib for 8 weeks and all studies were carried out during the mid-dark phase of the light cycle. All experiments were approved by the Institutional Animal Care and Research Advisory Committee of the University of Texas Southwestern Medical Center at Dallas.

Determination of HDL cholesteryl ether transport

Plasma was obtained from hamsters maintained on a standard low-cholesterol, low-fat rodent diet and HDL was isolated in the density range of 1.070–1.21 g/ml by sequential ultracentrifugation (20). The HDL was labeled with either the intracellularly trapped [1α,2α(n)-^{3}H]cholesteryl oleoyl ether (21, 22) or with [4-^{14}C]cholesteryl oleate by exchange from donor liposomes (15, 18, 23) and used within 24 h. Animals were administered a priming dose of [^{3}H]cholesteryl ether-labeled HDL through the femoral vein followed by a continuous infusion of the same radiolabeled lipoprotein so as to maintain a constant plasma specific activity (18). The infusions of [^{3}H]cholesteryl ether-labeled HDL were continued for 4 h at which time each animal was administered [^{14}C]cholesteryl ester-labeled HDL intravenously (as a marker of the volume of plasma in each tissue) and killed 10 min later. Plasma and tissue samples were saponified in alcoholic KOH (24) and the sterols were quantitatively extracted (24, 25) and assayed for ^{3}H and ^{14}C as previously described (18). The tissue spaces achieved by the labeled HDL at 10 min (^{14}C dpm per gram of tissue divided by the ^{14}C dpm per microliter of plasma) and at 4 h and 10 min (^{3}H dpm

per gram of tissue divided by the steady-state ^{3}H dpm per microliter of plasma) were then calculated and have the units of μl/g. The increase in tissue space over the 4-h experimental time period equals the rate of radiolabeled HDL cholesteryl ether movement into each organ and is expressed as the microliters of plasma cleared of its HDL cholesteryl ether content per hour per gram of tissue or per whole organ (18). Clearance values were multiplied by the plasma HDL cholesteryl ester concentration to obtain the absolute rates of HDL cholesteryl ester uptake expressed as the micrograms of HDL cholesteryl ester taken up per hour per gram of tissue or per whole organ. In some experiments the relationship between the rate of HDL cholesteryl ester transport and the concentration of HDL cholesteryl ester in plasma was determined by adding mass amounts of unlabeled hamster HDL (d 1.07–1.21 g/ml) to the primed infusions of trace-labeled HDL. The lipoprotein distribution of ^{3}H and ^{14}C was determined by FPLC (Pharmacia LKB Biotechnology, Uppsala, Sweden) using a Superose 6 (Sigma Chemical Co., St. Louis, MO) column as previously described (18).

Determination of HDL apoA-I transport

Hamster HDL (d 1.07–1.21 g/ml) was isolated as described above. Hamster HDL in this density range contains almost exclusively apoA-I (26); this was confirmed on overloaded SDS polyacrylamide gels (not shown). ApoA-I was labeled in situ with the residualizing marker ^{125}I-labeled tyramine cellobiose as originally described by Glass et al. (27) or with ^{131}I (28). HDL apoA-I transport was quantified in vivo using a primed continuous infusion of radiolabeled HDL apoA-I as previously described (18). Infusions were continued for 4 h at which time the animals were administered ^{131}I-labeled HDL intravenously (as a marker of the volume of plasma in each tissue) and killed 10 min later. Plasma and tissue samples were assayed for radioactivity in a gamma counter (Packard Instrument Co., Inc., Downers Grove, IL). The tissue spaces achieved by the labeled HDL at 10 min (^{131}I dpm per gram of tissue divided by the ^{131}I dpm per microliter of plasma) and at 4 h and 10 min (^{125}I dpm per gram of tissue divided by the steady-state ^{125}I dpm per microliter of plasma) were calculated and have the units of μl/g. The increase in tissue space over the 4-h experimental period equals the rate of radiolabeled HDL apoA-I movement into each organ and is expressed as the microliters of plasma cleared of its HDL apoA-I content per hour per gram of tissue or per whole organ (18). Clearance values were multiplied by the plasma HDL apoA-I concentration to obtain the absolute rates of HDL apoA-I uptake.

Determination of LDL cholesterol transport

Rates of LDL cholesterol uptake were measured using primed infusions of ^{125}I-labeled tyramine cellobiose-labeled hamster LDL as described previously (6). The infusions were continued for 4 h at which time each animal was administered a bolus of ^{131}I-labeled hamster LDL and killed 10 min later. Tissue samples and plasma were assayed for radioactivity and rates of LDL cholesterol uptake were calculated as described (6).

Determination of cholesterol synthesis rates

Hamsters were administered ~100 mCi of [^{3}H]water intravenously (24). One hour later the animals were anesthetized and exsanguinated through the abdominal aorta. Aliquots of plasma were taken for the determination of body water specific activity, and samples of liver and the entire remaining carcass were taken for the isolation of digitonin-precipitable sterols. Tissues were saponified and the digitonin-precipitable sterols were isolated and assayed for tritium content. Rates of sterol synthesis are expressed as the nmoles of [^{3}H]water incorporated into digitonin-precipitable sterols per hour per gram tissue or per whole tissue.

Determination of mRNA levels

Hepatic and small bowel apoA-I and hepatic scavenger receptor type BI (SR-BI) mRNA levels were determined using a nuclease protection assay as previously described (6). The level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA in the liver and intestine was not altered by the experimental diets and was used as an invariant control. Because Syrian hamster cDNA probes were not available for apoA-I and SR-BI, the polymerase chain reaction (PCR) was used, after reverse transcriptase synthesis of the complementary cDNA, to amplify partial cDNA fragments of apoA-I and SR-BI from Syrian hamster liver RNA. Oligonucleotide primers used to amplify a 648 bp partial apoA-I cDNA (5′-AAAGGATCCCTCCTGGACAACTGGGACA-3′, and 5′-AAAGAATTCTCAGGTGGTCGCTGGCCTTGG-3′) were selected from areas of 100% homology in the published rat and rabbit apoA-I sequences (29, 30). Oligonucleotide primers used to amplify a 380 bp partial SR-BI cDNA (5′-AAAGGATCCCGTGCCTTTATGAACCGA ACAGTT-3′, and 5′-AAAGAATTCCCTTCAAACACCCC TGAATCATGG-3′) were based on the published sequence for the Chinese hamster cDNA (31). These partial cDNA fragments were cloned into M13 for sequencing and the preparation of ^{32}P-labeled single-stranded probes. ^{32}P-labeled probes were synthesized as previously described using 0.5 μM [^{32}P]dCTP and 1 μM

(apoA-I and SR-BI) or 300 μM (GAPDH) unlabeled dCTP.

Samples of liver and small intestine were homogenized in guanidinium thiocyanate and the RNA was isolated by the method of Chomczynski and Sacchi (32). Total RNA (40 μg) was hybridized with the ^{32}P-labeled cDNA probes simultaneously at 48°C overnight. Unhybridized probe, present in excess relative to the amount of specific mRNA, was then digested with 40 units of mung bean nuclease (GIBCO BRL/Life technologies, Gaithersburg, MD). The mRNA-protected ^{32}P-labeled probes were separated on 7 M urea, 6% polyacrylamide gels together with ^{32}P-labeled *Msp*I-digested pBR322 size standards. The radioactivity in each band, as well as background radioactivity, was quantified using an isotopic imaging system (Ambis, Inc., San Diego, CA).

Determination of plasma cholesterol and apoA-I distribution

The cholesterol and apoA-I distributions in plasma lipoproteins were determined by FPLC as previously described (18). Cholesterol was assayed using an enzymatic kit (Sigma Chemical Co.). ApoA-I was assayed using a turbidometric assay (18, 33). The apoA-I antisera was prepared in rabbits using apoA-I purified from delipidated hamster HDL (d 1.07–1.21 g/ml) by anion exchange (Mono Q, Pharmacia, Inc., Piscataway, NJ) and gel filtration (superose 12) chromatography (18, 34).

Calculations and statistical analysis

The liver takes up HDL cholesteryl ester and apoA-I via saturable transport processes as recently described (18). Because HDL uptake by the liver is saturable, and because plasma HDL concentrations varied considerably among the experimental groups, changes in HDL transport could not be directly equated with regulation of the activity of the HDL transport process. To determine whether a change in HDL apoA-I or cholesteryl ether clearance was due to regulation of an HDL transport mechanism or simple reflected a change in the concentration of particles competing for this transport mechanism, rates of hepatic HDL cholesteryl ether and apoA-I transport were superimposed on kinetic curves (±95% confidence intervals) that describe the relationship between HDL transport and circulating HDL concentrations in the control hamster. The kinetic parameters for HDL apoA-I and cholesteryl ether transport were previously determined by quantifying rates of HDL cholesteryl ether and apoA-I transport in control hamsters under conditions in which circulating HDL concentrations were acutely raised and maintained at various levels during the 4-h period of time over which HDL transport was measured (18). By relating rates of HDL cholesteryl ether transport in the experimental animals to these normal kinetic curves, it was possible to determine how the experimental diets affected transporter activity.

The data are presented as means ± 1 SD. To test for differences among dietary regimens, one-way analysis of variance was performed using the statistical software package StatView 4.5 (Abacus Concepts, Berkeley, CA) for the Macintosh. Significant effects were further analyzed using Sheffe's F procedure for post-hoc comparisons.

RESULTS

Diet-induced alterations in plasma and liver cholesterol levels

Hamsters were fed diets previously shown to significantly alter plasma HDL cholesterol concentrations (6). The lipoprotein distribution of plasma cholesterol in animals fed the control, psyllium-supplemented or Western-type diet is shown in **Fig. 1.** LDL and HDL cholesterol concentrations increased in animals consuming the Western-type diet and decreased in animals fed the

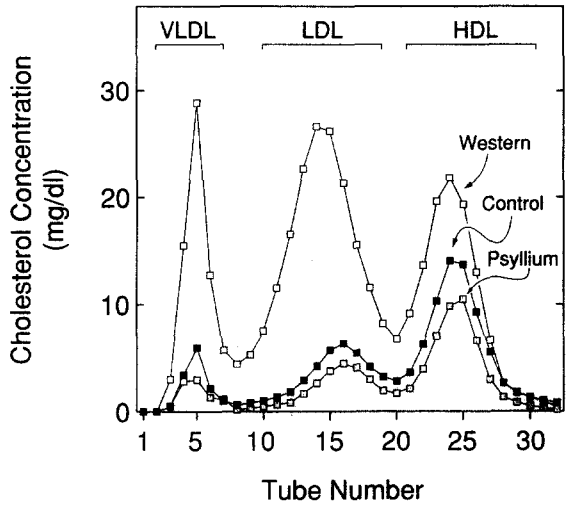

Fig. 1. Distribution of plasma lipoprotein cholesterol by particle size in animals consuming control, psyllium-supplemented, or Western-type diets. Animals were fed a control low-cholesterol, low-fat rodent diet or the same diet supplemented with 5% psyllium or a mixture of cholesterol and triglycerides intended to mimic a Western-type diet. After 8 weeks on the experimental diets, animals were killed and equal volumes of plasma from 6 animals were pooled. Lipoproteins were separated by FPLC using a Superose 6 column as described in Methods. Two-ml aliquots were collected and assayed for cholesterol. Each point represents the mean value from two experiments in which plasma was pooled from 6 animals per group. The retention times for hamster VLDL (d < 1.006 g/ml), LDL (d 1.02–1.055 g/ml), and HDL (d 1.07–1.21 g/ml) are indicated.

psyllium-supplemented diet. Plasma HDL cholesterol concentrations in animals fed the Western-type diet were 46% higher than in animals fed the control diet and 86% higher than in animals fed the psyllium-supplemented diet. Diet-related changes in plasma HDL cholesterol concentrations were mainly due to alterations in the amount of cholesterol carried in larger HDL particles (Fig. 1). In all diet groups, the majority of HDL cholesterol was esterified, with cholesterol esters accounting for 86–91% of total cholesterol in tubes corresponding to HDL (tubes 21–30). The cholesterol concentration of the liver equaled 2.7 mg/g, 2.4 mg/g, and 22 mg/g in animals fed the control, psyllium-supplemented, and Western-type diets, respectively.

Diet-induced alterations in HDL cholesteryl ester transport

Rates of HDL cholesteryl ester transport were quantified in vivo using homologous HDL labeled with [^{3}H]cholesteryl oleyl ether, a nondegradable tracer of cholesteryl esters that remains intracellularly trapped following uptake (18). As shown in **Fig. 2,** very little HDL cholesteryl ether accumulated in the lower density lipoprotein fractions during the 4-h infusions, consistent with the known low level of CETP in this species (26, 35). At the completion of the 4-h infusion, the amount of radiolabel present in LDL was slightly greater in animals fed psyllium whereas the amount of radiolabel in VLDL was slightly greater in animals fed the Western-type diet. **Table 1** shows rates of tissue HDL cholesteryl ether clearance (top) and cholesteryl ester uptake (bottom) in animals consuming the control or experimental diets. Clearance of HDL cholesteryl ether by the liver, which is almost entirely mediated by a receptor-dependent (saturable) process(es) (18), varied inversely with plasma HDL cholesteryl ester concentrations. As a consequence, the absolute rate of HDL cholesteryl ester uptake (the rate of HDL cholesteryl ether clearance times the plasma HDL cholesteryl ester concentration) by the liver was constant under conditions in which plasma HDL cholesteryl ester concentrations varied by nearly 2-fold.

The inverse relationship between hepatic HDL cholesteryl ether clearance and plasma HDL cholesteryl ester concentrations could be the result of diet-induced alterations in the activity of the transport mechanism(s) or simply due to changes in the concentration of particles competing for these transporters. To quantify changes in transporter activity in vivo, rates of HDL cholesteryl ester transport in the experimental animals were related to kinetic curves describing the relationship between HDL cholesteryl ester transport and circulating HDL cholesteryl ester concentrations in control animals. **Figure 3** shows the kinetic curves for hepatic

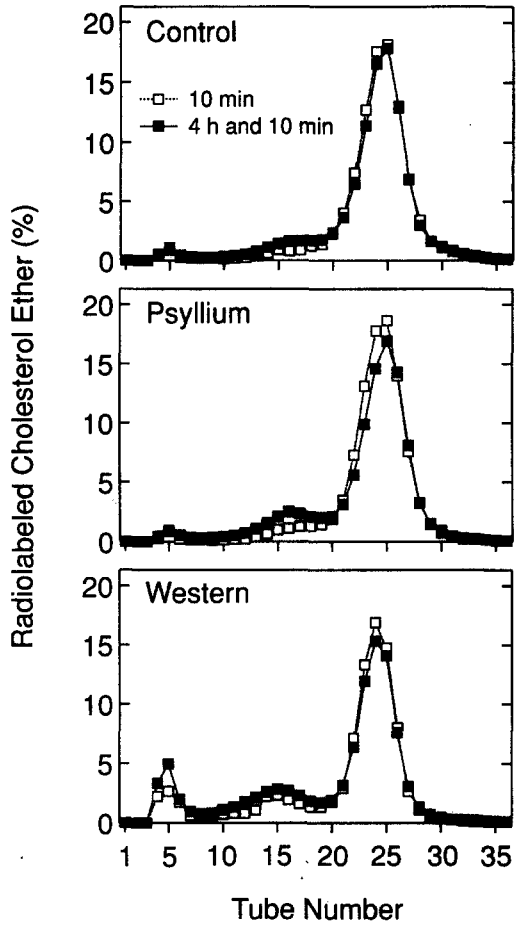

Fig. 2. Lipoprotein distribution of radiolabeled cholesteryl ether as a function of time in animals administered primed infusions of [^{3}H]cholesteryl oleyl ether-labeled HDL. Plasma was obtained from animals 10 min or 4 h and 10 min (250 min) after initiation of the primed infusions and equal volumes from each animal were pooled and separated by FPLC as described in Methods. Two-ml fractions were collected and assayed for radioactivity. The retention times for hamster VLDL (d < 1.006 g/ml), LDL (d 1.02–1.055 g/ml), and HDL (d 1.07–1.21 g/ml) are indicated. The data represent the mean of two experiments in which plasma was pooled from 6 animals per experimental group.

HDL cholesteryl ester transport in the control hamster. These kinetic curves (±95% confidence intervals) were previously determined by quantifying rates of HDL cholesteryl ester uptake in control hamsters under conditions in which circulating HDL concentrations were acutely raised and maintained at various levels during the 4-h experimental period (18). Rates of hepatic HDL cholesteryl ether clearance (top) and HDL cholesteryl ester uptake (bottom) in animals fed the experimental diets are superimposed on these normal kinetic curves.

In the control animals, HDL cholesteryl ether clearance equaled 74 ± 9 μl/h per g liver and the absolute rate of HDL cholesteryl ester uptake equaled 48 μg/h

TABLE 1. HDL cholesteryl ester transport in animals with diet-induced alterations in plasma HDL levels

Diet	Liver	Small Bowel	Adrenal Glands	Spleen	Remaining Carcass	Whole Body
			μl/h per whole tissue per 100 g body wt			
Clearance						
Control	278 ± 44	6.9 ± 1	3.4 ± 0.6	1.3 ± 0.1	80 ± 12	370 ± 61
Psyllium	349 ± 62	6.7 ± 1	5.8 ± 1.4	1.5 ± 0.4	82 ± 13	445 ± 69
Western	194 ± 36	5.3 ± 2	2.4 ± 0.8	1.2 ± 0.2	72 ± 10	275 ± 44
			μg/h per whole tissue per 100 g body wt			
Uptake						
Control	181 ± 31	4.5 ± 1	2.2 ± 0.4	0.85 ± 0.1	52 ± 7	241 ± 35
Psyllium	178 ± 35	3.4 ± 1	3.0 ± 0.8	0.77 ± 0.2	42 ± 4	227 ± 26
Western	184 ± 40	5.0 ± 2	2.3 ± 0.8	1.1 ± 0.2	68 ± 6	265 ± 31

Each value represents the mean ± 1 SD for data obtained in 12 animals. Rates of HDL cholesteryl ether clearance were quantified as described in Methods. Tissue clearance rates were multiplied by the plasma concentration of HDL cholesteryl ester in the same animal to yield the absolute rate of HDL cholesteryl ester uptake. Mean plasma HDL cholesteryl ester concentrations equaled 65 ± 6, 51 ± 7, and 95 ± 12 mg/dl in the control, psyllium-supplemented, and Western-type diet groups, respectively.

per g liver at a plasma HDL cholesteryl ester concentration of 65 mg/dl. These values fell on the kinetic curves describing the relationship between HDL cholesteryl ester transport and plasma HDL cholesteryl ester concentrations in the normal hamster. Similarly, the values for HDL cholesteryl ester transport in animals consuming the psyllium-supplemented and Western-type diets were not displaced from the kinetic curves for HDL transport in the control hamster. We also measured rates of HDL cholesteryl ester transport in a group of psyllium-fed animals under conditions in which mass amounts of HDL (obtained from hamsters fed the Western-type diet) were added to the primed infusions of trace-labeled HDL to acutely raise and maintain plasma HDL cholesterol concentrations at values similar to those present in animals fed the Western-type diet. When measurements of HDL cholesteryl ester transport in animals fed the psyllium-supplemented and Western-type diets were performed at the same plasma HDL cholesteryl ester concentration, rates of hepatic HDL cholesteryl ether clearance and HDL cholesteryl ester uptake were similar in the two groups.

To determine whether HDL from the three diet groups competes similarly for hepatic uptake, HDL cholesteryl ester transport was measured in control animals under conditions in which mass amounts of HDL from animals fed the control or experimental diets were added to the primed infusions. As shown in **Fig. 4,** hepatic HDL cholesteryl ether clearance equaled 72 μl/h per g in control animals at a plasma HDL cholesteryl ester concentration of 68 mg/dl. When mass amounts of HDL were added to the primed infusion of trace-labeled HDL, the decrease in hepatic HDL clearance was similar whether the HDL mass was obtained from animals fed the control, psyllium-supplemented, or Western-type diets.

The exact nature of the transporters responsible for HDL cholesteryl ester uptake is under active investigation. SR-BI has been shown to mediate selective HDL cholesteryl ester uptake in CHO cells (36). The studies outlined above suggest no change in the activity of hepatic HDL cholesteryl ester transporters. We therefore examined the effect of the experimental diets on the hepatic expression of SR-BI using a nuclease protection assay and probes specific for the Syrian hamster. **Figure 5** is an example of an autoradiogram from a nuclease protection analysis of hepatic SR-BI mRNA levels in animals fed the experimental diets. When the data from 12 animals per group were quantified using an isotopic image analysis system as described in Methods, hepatic SR-BI mRNA levels were the same in animals fed the control (100 ± 11%), psyllium-supplemented (97 ± 19%), and Western-type diets (98 ± 5%).

A change in the plasma concentration of HDL cholesteryl ester can be due to a change in the rate at which cholesteryl esters enter plasma HDL or to a change in the rate at which cholesteryl esters are removed from the HDL fraction. As shown in Table 1 whole body HDL cholesteryl ester transport equaled 241 ± 35, 227 ± 26, and 265 ± 31 μg/h in the control, psyllium-supplemented, and Western-type diet groups, respectively. Although these changes were relatively small, the difference in whole body HDL cholesteryl ester transport between the psyllium-supplemented and Western-type diets (17% higher on the Western-type diet) was statistically significant ($P < 0.05$). This observation suggests that diet-induced changes in HDL cholesteryl ester concentrations were due, at least in

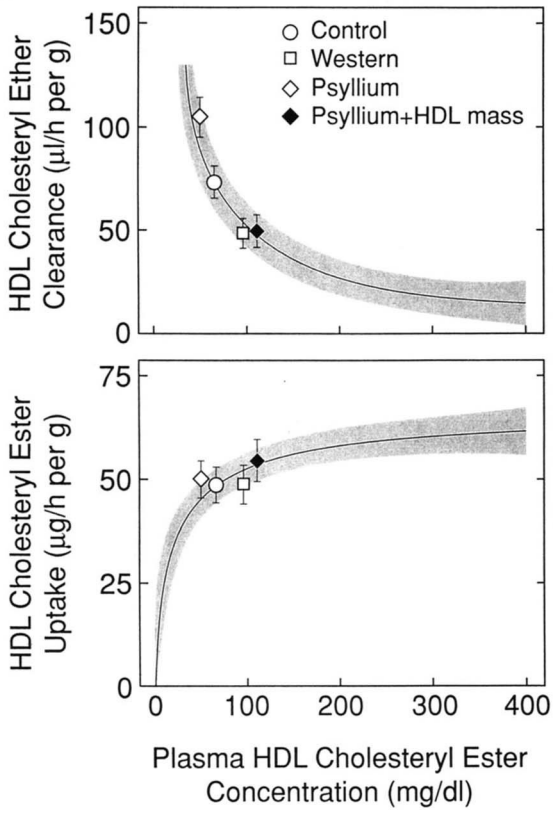

Fig. 3. Effect of diet modification on HDL cholesteryl ester transport in the liver. Groups of animals were fed a control low-cholesterol, low-fat diet or the same diet supplemented with psyllium, or a mixture of cholesterol and triglycerides intended to mimic a Western-type diet. Clearance rates for [^{3}H]cholesteryl ether-labeled homologous HDL were measured in vivo as described in Methods. These clearance rates were multiplied by the plasma HDL cholesteryl ester concentration in each animal to obtain the absolute rate of HDL cholesteryl ester uptake. The shaded areas represent the kinetic curves (±95% confidence intervals) for HDL cholesteryl ester transport in control animals. Superimposed on these kinetic curves are the rates of HDL cholesteryl ether clearance (top) and HDL cholesteryl ester transport (bottom) in the experimental animals plotted as a function of the plasma HDL cholesteryl ester concentration in the same animals. HDL cholesteryl ester transport rates were also measured in a group of animals fed the psyllium-supplemented diet under conditions in which plasma HDL cholesterol concentrations were acutely raised and maintained at levels similar to those in the Western-type diet group by adding mass amounts of HDL (obtained from hamsters fed the Western-type diet) to the primed infusions of trace-labeled HDL. Each point represents the mean for data obtained in 12 (control, psyllium-supplemented and Western-type diet groups) or 4 (psyllium-supplemented diet plus HDL mass group) animals.

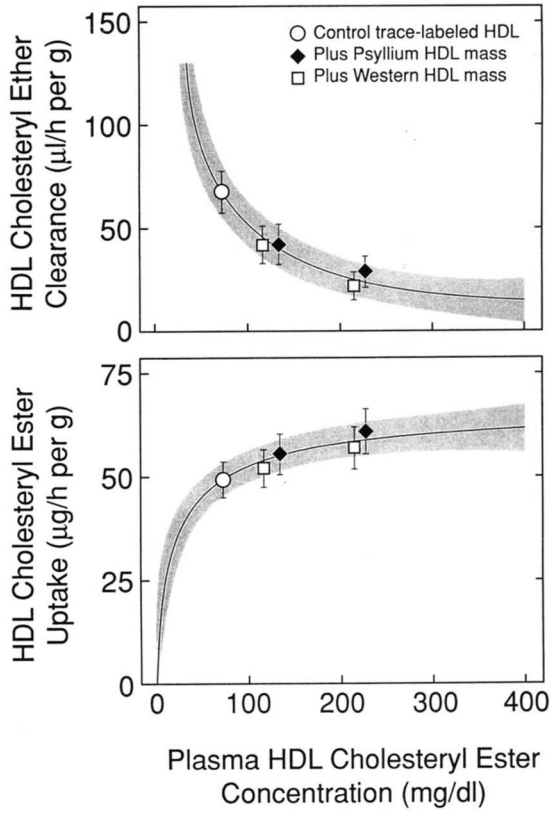

Fig. 4. Effect of HDL from animals fed the psyllium-supplemented or Western-type diets on HDL cholesteryl ester transport. Rates of HDL cholesteryl ester transport were measured in control animals and in control animals infused with mass amounts of HDL from animals fed the psyllium-supplemented or Western-type diets. The shaded areas represent the kinetic curves (±95% confidence intervals) for HDL cholesteryl ester transport in control animals. Superimposed on these kinetic curves are the rates of HDL cholesteryl ether clearance (top) and HDL cholesteryl ester transport (bottom) in the experimental animals plotted as a function of the plasma HDL cholesteryl ester concentration in the same animals. Each value represents the mean ± 1 SD for data obtained in 6 (trace-labeled HDL only) or 4 (trace-labeled HDL plus mass amounts of HDL from animals fed the psyllium or Western diets) animals.

part, to an increase in the rate of cholesteryl ester entry into the HDL fraction.

In a steady state, the bulk of the cholesterol that is acquired by extrahepatic tissues (from lipoprotein uptake or de novo synthesis) must be returned to the liver for excretion. In the control hamster, the rate of HDL cholesteryl ester uptake by the liver closely approximates the rate at which cholesterol is acquired by the extrahepatic tissues (from LDL, HDL, and de novo synthesis) suggesting that hepatic HDL cholesteryl ester uptake is a reasonable reflection of reverse cholesterol transport (18). To determine whether a similar situation exists under conditions in which hepatic sterol balance and plasma lipoprotein concentrations are markedly altered, we quantified rates of LDL cholesterol uptake and de novo cholesterol synthesis in all extrahepatic tissues of animals fed the control, psyllium-supplemented, or Western-type diets.

Rates of LDL transport were quantified in vivo using ^{125}I-labeled tyramine cellobiose-labeled hamster LDL and a primed infusion protocol as described in Methods. The extrahepatic tissues cleared LDL cholesterol at rates of 92 ± 12, 102 ± 14, and 37 ± 4 µl/h per 100

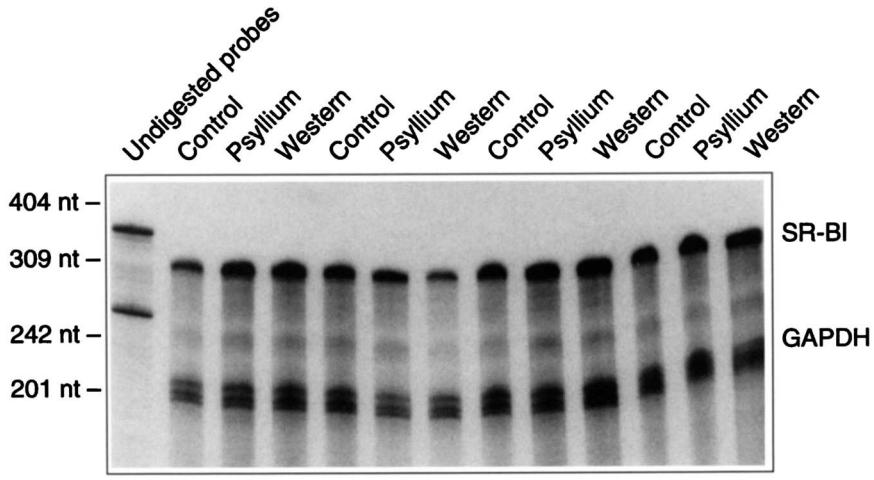

Fig. 5. Measurement of hepatic SR-BI mRNA levels. Hepatic mRNA was isolated from animals fed the control or experimental diets. Total RNA (40 μg) was hybridized with ^{32}P-labeled single-stranded cDNA probes and the protected bands resistant to mung bean nuclease digestion were analyzed by polyacrylamide gel electrophoresis followed by autoradiography.

g body wt in animals fed the control, psyllium-supplemented, and Western-type diets, respectively. Tissue clearance rates were multiplied by the plasma LDL cholesterol concentration (mean values equaled 26 ± 4, 19 ± 3, and 133 ± 13 mg/dl in the control, psyllium-supplemented, and Western-type diet groups, respectively) to obtain the mass of LDL cholesterol transported by the extrahepatic tissues. As shown in **Table 2** the extrahepatic tissues took up LDL cholesterol at rates of 24 ± 3, 19 ± 3, and 49 ± 5 μg/h per 100 g body wt in animals fed the control, psyllium-supplemented, and Western-type diets, respectively.

Rates of cholesterol synthesis in the liver and extrahepatic tissues were measured in vivo using [^{3}H]water. Consistent with previous studies, rates of hepatic cholesterol synthesis were suppressed in animals fed the Western-type diet and increased in animals fed the psyllium-supplemented diet (data not shown). As shown in Table 2, rates of cholesterol synthesis were also lower in the extrahepatic tissues of animals fed the Western-type diet (98 ± 9 μg/h) than in animals fed the control (114 ± 15 μg/h) or psyllium-supplemented (135 ± 11 μg/h) diets due mainly to the increased amounts of LDL and HDL cholesterol that were taken up by these tissues in animals fed the Western-type diet.

Rates of total cholesterol acquisition by the extrahepatic tissues (from LDL cholesterol uptake, de novo cholesterol synthesis, and HDL cholesteryl ester uptake) are shown in Table 2 and equaled 198, 203, and 223 μg/h in animals fed the control, psyllium-supplemented, and Western-type diets, respectively. The small increase in cholesterol acquisition by the extrahepatic

TABLE 2. Cholesterol acquisition in the extrahepatic tissues and hepatic HDL cholesteryl ester uptake in animals with diet-induced alterations in plasma HDL levels

	Cholesterol Acquisition by Extrahepatic Tissues				
Diet	LDL Cholesterol Uptake	De novo Synthesis	HDL Cholesteryl Ester Uptake	Total Cholesterol Aquisition	Hepatic HDL Cholesterol Ester Uptake
	μg/h per whole tissue per 100 g body wt				
Control	24 ± 3	114 ± 15	60 ± 10	198	181 ± 31
Psyllium	19 ± 3	135 ± 11	49 ± 6	203	178 ± 35
Western	49 ± 5	98 ± 9	76 ± 12	223	184 ± 40

Values represent the mean ± 1 SD for data obtained in 12 (LDL cholesterol and HDL cholesteryl ester uptake) or 6 (cholesterol synthesis) animals. Rates of cholesterol synthesis are expressed as the μg of sterol formed per hour assuming that 22 ^{3}H are incorporated into the cholesterol molecule during synthesis from acetyl-CoA in the presence of [^{3}H]water in vivo (24).

tissues of animals fed the Western-type diet was due to the increased rates of HDL and LDL cholesterol uptake that were not fully balanced by the decrease in de novo cholesterol synthesis. In contrast to cholesterol acquisition by the extrahepatic tissues, which appeared to be modestly increased in animals fed the Western-type diet, rates of hepatic HDL cholesteryl ester uptake were the same in animals fed the control, psyllium-supplemented, and Western-type diets.

Diet-induced alterations in HDL apoA-I transport

Changes in plasma HDL apoA-I concentrations paralleled those of HDL cholesteryl ester but were smaller in magnitude as shown in **Fig. 6.** The mean plasma HDL apoA-I concentration in animals consuming the Western-type diet was 25% higher than in the control animals and 45% higher than in animals fed the psyllium-supplemented diet. As shown in **Table 3,** rates of hepatic HDL apoA-I clearance were not altered under conditions in which plasma HDL apoA-I concentrations varied from 97 mg/dl to 141 mg/dl in response to diet modification. Similarly, with the exception of the small bowel, rates of HDL apoA-I clearance were minimally altered in the extrahepatic tissues or whole body in response to diet modification. As a consequence, the absolute rate of HDL apoA-I uptake varied in proportion to the change in plasma HDL apoA-I concentrations. Whole body HDL apoA-I uptake was significantly ($P < 0.05$) higher in animals fed the Western-type diet (226 ± 36 μg/h) than in animals fed the control (189 ± 26 μg/h) or psyllium-supplemented (167 ± 22 μg/h) diets. Because the rate of HDL apoA-I uptake by all tissues of the body must equal the rate of apoA-I entry into HDL, these data suggest that changes in the rate of apoA-I entry into the HDL fraction are largely responsible for diet-induced changes in plasma HDL apoA-I concentrations.

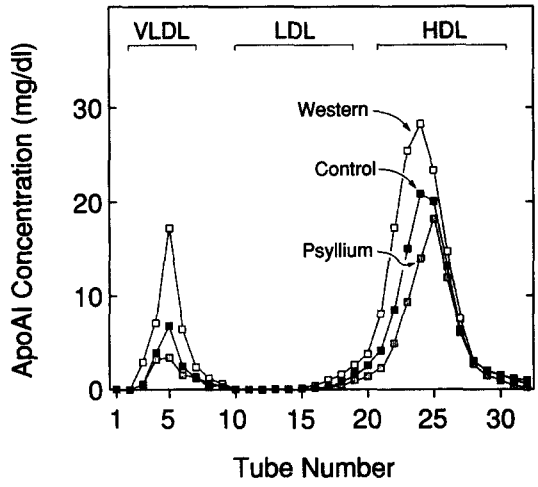

Fig. 6. Distribution of plasma lipoprotein apoA-I by particle size in animals consuming control, psyllium-supplemented, or Western-type diets. Plasma lipoproteins from the same groups of animals described in Fig. 1 were separated by FPLC and each fraction was assayed for apoA-I.

Plasma apoA-I is synthesized and secreted exclusively by the liver and small intestine. ApoA-I mRNA levels were therefore quantified by nuclease protection in the liver and small intestine of animals with diet-induced alterations in plasma apoA-I concentrations. These studies showed little effect of the experimental diets on hepatic or intestinal apoA-I mRNA levels. Additional studies were therefore performed comparing animals

TABLE 3. HDL apoA-I transport in animals with diet-induced alterations in plasma HDL levels

Diet	Liver	Small Bowel	Adrenal Glands	Spleen	Remaining Carcass	Whole Body
	μl/h per whole tissue per 100 g body wt					
Clearance						
Control	51 ± 7	6.4 ± 1	19 ± 2	11 ± 2	80 ± 12	167 ± 24
Psyllium	54 ± 8	9.2 ± 2	17 ± 6	10 ± 1	82 ± 6	172 ± 19
Western	54 ± 8	5.2 ± 2	20 ± 3	9 ± 1	72 ± 10	160 ± 27
	μg/h per whole tissue per 100 g body wt					
Uptake						
Control	58 ± 8	7.2 ± 1	21 ± 3	12 ± 2	90 ± 11	189 ± 26
Psyllium	52 ± 6	9 ± 2	16 ± 2	10 ± 2	80 ± 7	167 ± 22
Western	76 ± 9	7.3 ± 1	28 ± 5	13 ± 1	102 ± 13	226 ± 36

Each value represents the mean ± 1 SD for data obtained in 12 animals. Rates of HDL apoA-I clearance were quantified as described in Methods. Tissue clearance rates were multiplied by the plasma concentration of HDL apoA-I in the same animal to yield the absolute rate of HDL apoA-I uptake. Mean plasma HDL apoA-I concentrations equaled 113 ± 16, 97 ± 14, and 141 ± 18 mg/dl in the control, psyllium-supplemented, and Western-type diet groups, respectively.

TABLE 4. ApoA-I mRNA levels in the liver and small bowel in animals with diet-induced alterations in plasma HDL levels

Diet	Liver	Small Bowel	
		Proximal	Distal
Psyllium	100 ± 13	100 ± 22	100 ± 12
Western	118 ± 9[a]	96 ± 29	103 ± 14

Values represent the mean ± 1 SD for data obtained in 12 animals: ApoA-I mRNA levels were determined by nuclease protection as described in Methods. Values in animals fed the Western diet are expressed as a percentage of those in the psyllium diet.

[a]Differs significantly from the psyllium value ($P < 0.05$).

with the highest (Western-type diet) and lowest (psyllium-supplemented diet) HDL apoA I levels. As shown in **Table 4,** when the nuclease protection analyses from 12 animals (in each group) were quantified on an isotopic image analysis system as described in Methods, there was a small but statistically significant increase in hepatic apoA-I mRNA levels in animals consuming the Western-type diet relative to animals fed the psyllium-supplemented diet (18%, $P < 0.05$). Small bowel apoA-I mRNA levels were not altered by the diets used in these studies and were similar in proximal and distal regions.

DISCUSSION

The major finding of the current work is that diet-induced alterations in plasma HDL cholesterol concentrations are not necessarily accompanied by changes in the transport of HDL cholesteryl ester to the liver. Although plasma HDL cholesteryl ester concentrations were nearly 50% lower in hamsters fed a low-fat, high-fiber diet than in animals fed a Western-type diet, the flux of HDL cholesteryl esters to the liver was the same in the two groups. Previous studies in the hamster have shown that the flux of HDL cholesteryl esters to the liver equals the rate of cholesterol acquisition by the extrahepatic tissue compartment and, as such, accurately reflects the rate of reverse cholesterol transport (18). Together, these studies indicate that a low-fat, high-fiber diet may lower the plasma concentration of HDL cholesterol but does not adversely affect the rate of reverse cholesterol transport, at least in the hamster. It should be noted that diet-induced changes in the plasma concentration of HDL could affect atherogenesis via mechanisms independent of reverse cholesterol transport (37, 38). However, whereas epidemiological studies show an inverse relationship between plasma HDL concentrations and clinical coronary heart disease in populations consuming a Western diet (1, 2), there is little evidence that the decrease in the plasma concentration of HDL that frequently accompanies a cholesterol-lowering diet adversely affects atherogenesis. Indeed, coronary artery atherosclerosis was found to be less in nonhuman primates fed a polyunsaturated fat diet than in animals fed a saturated fat diet even though the polyunsaturated fat diet produced equivalent reductions in LDL (39%) and HDL (36%) cholesterol concentrations (39).

We previously showed that HDL cholesteryl ester uptake by the liver is largely saturated at normal HDL concentrations (18). Based on these findings it could be predicted that the absolute rate of HDL cholesteryl ester uptake by the liver can only be altered when HDL concentrations are drastically reduced or when the transport process is regulated. In the current studies, rates of hepatic HDL cholesteryl ester transport in animals fed the psyllium-supplemented or Western-type diets were not displaced from the kinetic curves for HDL cholesteryl ester transport in the control hamster. Moreover, when rates of HDL cholesteryl ester transport in animals fed the psyllium-supplemented diet were measured under conditions in which plasma HDL cholesterol concentrations were acutely raised and maintained at levels equivalent to those of animals fed the Western-type diet, rates of hepatic HDL cholesteryl ether clearance and HDL cholesteryl ester uptake were the same in the two groups. Although plasma HDL cholesteryl ether clearance rates (equivalent to FCRs) varied inversely with plasma HDL cholesteryl ester concentrations, these changes reflect differences in the plasma concentration of particles competing for the HDL cholesteryl ester transport process(es) rather than regulation of the number or affinity of these transporters. This conclusion is further supported by the finding that hepatic mRNA levels of SR-BI did not differ among animals fed the control, psyllium-supplemented, and Western-type diets.

The precise role of SR-BI in hepatic HDL cholesteryl ester transport has not been defined; nevertheless, this receptor is capable of mediating selective HDL cholesteryl ester uptake when transfected into LDL receptor negative CHO cells (36) and is coordinately regulated with steroidogenesis in the adrenal gland, ovaries, and testes (40, 41). This latter observation suggests that HDL cholesteryl ester uptake and SR-BI expression may be subject to feedback control by cholesterol in these tissues. This clearly is not the case in the liver as no regulation of receptor-dependent HDL cholesteryl ester uptake or of SR-BI expression was observed under conditions in which the cholesterol content of the liver varied by 10-fold. These data are consistent with previous observations in primary rat hepatocyte cultures that showed no regulation of HDL apoA-I or cholesteryl ester uptake in response to prior cholesterol loading (42).

In contrast, regulation of selective HDL cholesteryl ester uptake in primary hepatocyte cultures from fasted or cholestyramine-fed rabbits has been reported (43) as has sterol-mediated regulation in a variety of cultured cell lines including the liver-derived HepG2 cell line (42, 44). The reason for these seemingly inconsistent observations is, at present, unclear.

Although diet modification did not alter the transport of HDL cholesteryl esters to the liver, plasma HDL cholesteryl ester concentrations were nearly 2-fold higher in animals fed the Western-type diet than in animals fed the psyllium-supplemented diet. An increase in the plasma concentration of HDL cholesteryl ester can be due to an increase in the rate of cholesteryl ester entry into the HDL fraction, a decrease in the rate of cholesteryl ester removal from this pool, or a combination of both. Rates of hepatic HDL cholesteryl ether clearance and HDL cholesteryl ester uptake were not displaced from the normal kinetic curves for HDL cholesteryl ester transport regardless of the experimental diet indicating no regulation of the receptor dependent removal process(es). On the other hand, whole body HDL cholesteryl ester transport was 17% higher in animals fed the Western-type diet than in animals fed the psyllium-supplemented diet due to an increase in HDL cholesteryl ester uptake in the extrahepatic tissues. As cholesteryl ester must enter and leave HDL at the same rate under steady-state conditions, these data indicate that the change in HDL cholesteryl ester concentration was due to a change in the rate of cholesteryl ester entry into HDL (whole body HDL cholesteryl ester transport). Because HDL cholesteryl ester removal from plasma is largely via a receptor-dependent process that is saturated at normal HDL concentrations, small changes in the rate of cholesteryl ester entry into the HDL fraction will produce disproportionately large changes in circulating HDL cholesteryl ester levels.

In a steady state, the bulk of the cholesterol that is acquired by extrahepatic tissues must be returned to the liver for excretion. In animals fed the control and psyllium-supplemented diets, the rate of cholesterol acquisition by the extrahepatic tissues was approximately balanced by an equivalent flux of HDL cholesteryl ester to the liver. In animals fed the Western-type diet, cholesterol acquisition by the extrahepatic tissue compartment was increased ~10% (compared to the control or psyllium-fed groups) due to an increase in HDL and LDL cholesterol uptake that was not fully balanced by repression of de novo cholesterol synthesis. In contrast to cholesterol acquisition by the extrahepatic tissues, which appeared to be increased in animals fed the Western-type diet, the transport of HDL cholesteryl esters to the liver was identical in animals fed the control, psyllium-supplemented, and Western-type diets. Consequently, the small increase in cholesterol acquisition by the extrahepatic tissues (~20 μg/h) in animals fed the Western-type diet would eventually lead to an increased cholesterol content in the extrahepatic tissues if no other compensatory mechanisms are called into play. In other studies we have found that consumption of a hypercholesterolemic diet does lead to a small but significant increase in the cholesterol content of the extrahepatic tissue compartment in the hamster (L. A. Woollett and D. K. Spady, unpublished observation). Moreover, the cholesterol content of the aorta clearly increases in hamsters fed hypercholesterolemic diets (45).

The dietary factors we examined disproportionately affected the plasma concentration of larger (and presumably lighter) HDL particles. Although we did not systematically evaluate the kinetic properties of specific HDL subfractions, we did test the ability of HDL from animals fed the psyllium-supplemented or Western-type diets to compete with control HDL for tissue cholesteryl ester uptake. In these studies, rates of HDL cholesteryl ether clearance were measured in control animals infused with mass amounts of unlabeled HDL obtained from animals fed the psyllium-supplemented or Western-type diets. Under these conditions, a given increase in the concentration of HDL cholesteryl ester was associated with a similar reduction in the hepatic clearance of HDL cholesteryl ether regardless of whether the HDL was derived from animals fed control, psyllium-supplemented, or Western-type diets. These observations suggest that no major differences exist among HDL subfractions with respect to the kinetics of HDL cholesteryl ester transport in the hamster in vivo. These data are consistent with observations in a mouse adrenal cell line, which showed that rates of HDL cholesteryl ester uptake were similar for various HDL subfractions, although the ratio of particle to selective uptake varied somewhat (46).

HDL apoA-I concentrations were ~45% higher in animals fed the Western-type diet than in animals fed the psyllium-supplemented diet. As was the case with HDL cholesteryl ester, diet-induced changes in HDL apoA-I concentrations were due entirely to changes in the rate of apoA-I entry into HDL (whole body HDL apoA-I transport). These observations are consistent with apoA-I turnover data in mice (47), rabbits (48), and humans (7), which showed that diets rich in saturated fat and cholesterol significantly increase the rate of apoA-I production (whole body apoA-I transport rate). In the current studies, diet-induced changes in plasma apoA-I concentrations were associated with small changes in hepatic, but not intestinal, apoA-I mRNA levels. Changes in steady-state apoA-I mRNA levels were considerably less than the changes in apoA-I production

suggesting that much of the regulation in apoA-I production occurred at the posttranscriptional level, as previously noted in the human apoA-I transgenic mouse model, where the principal effect of dietary saturated fat and cholesterol was on the translatability of hepatic apoA-I mRNA (49). Studies in nonhuman primates also suggest a major effect of dietary fat on apoA-I production; however, in these studies regulation appeared to be exerted primarily at the level of steady-state apoA-I mRNA levels (50, 51).

Numerous studies have examined HDL turnover in humans or in animal models. In many of these studies, changes in HDL concentrations were associated with changes in the FCR of HDL apoA-I (47, 52–54) or cholesteryl ester (47) suggesting regulation of a receptor dependent clearance mechanism(s). Based on the present studies, it is likely that these changes in FCR reflect the kinetic characteristics of the transport process and the fact that the transport process is largely saturated at normal HDL concentrations. As illustrated in Fig. 3, the clearance (equivalent to FCR) of HDL cholesteryl ether is highly sensitive to plasma HDL concentrations such that an acute change in HDL concentrations in normal animals results in reciprocal changes in the clearance (or FCR) of HDL cholesteryl ether due to competition for a limited number of transport sites. Furthermore, because HDL cholesteryl ester transport is largely via a receptor-dependent pathway that is saturated at normal HDL concentrations, small changes in the rate of HDL cholesteryl ester entry into the HDL fraction (whole body HDL cholesteryl ester transport) can result in relatively large changes in circulating HDL levels. These findings emphasize the importance of knowing the relationship between plasma HDL concentrations and rates of HDL transport in normal animals. Without knowledge of the kinetics of normal HDL transport, it is impossible to interpret the results of transport data under experimental conditions in which plasma HDL concentrations are altered.

In summary, these studies show that diet-induced alterations in plasma HDL cholesterol concentrations are not necessarily accompanied by parallel changes in the transport of HDL cholesteryl esters to the liver. This observation is consistent with studies in the CETP transgenic mouse, where a marked reduction in the plasma concentration of HDL cholesterol was not accompanied by a change in the rate at which the extrahepatic tissues acquired cholesterol from de novo synthesis or LDL uptake (11). In addition, these studies demonstrate that diet-induced alterations in plasma HDL cholesteryl ester and apoA-I concentrations are not due to regulation of HDL cholesteryl ester and apoA-I clearance mechanisms but rather are the result of changes in the rate at which these moieties enter the HDL fraction. Whereas changes in the rate of apoA-I entry into plasma HDL likely reflect changes in apoA-I synthesis and secretion by the liver and/or intestine, the source of the cholesteryl ester cannot be determined from the current studies. Nor is it clear how production of the surface and core constituents of HDL (which may enter and leave the particle independently) is integrated.

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