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Research Article

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Regulation of Hepatic 7α-hydroxylase Expression by Dietary Psyllium in the Hamster

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Abstract

Soluble fiber consistently lowers plasma total and low density lipoprotein (LDL)-cholesterol concentrations in humans and various animal models including the hamster; however, the mechanism of this effect remains incompletely defined. We performed studies to determine the activity of dietary psyllium on hepatic 7α-hydroxylase, 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase and LDL receptor expression in the hamster. In animals fed a cholesterol-free semisynthetic diet containing 7.5% cellulose (avicel) as a fiber source, substitution of psyllium for avicel increased hepatic 7α-hydroxylase activity and mRNA levels by 3–4-fold. Comparable effects on 7α-hydroxylase expression were observed with 1% cholestyramine. Psyllium also increased hepatic 7α-hydroxylase activity and mRNA in animals fed a diet enriched with cholesterol and triglyceride. Activation of 7α-hydroxylase was associated with an increase in hepatic cholesterol synthesis that was apparently not fully compensatory since the cholesterol content of the liver declined. Although dietary psyllium did not increase hepatic LDL receptor expression in animals fed the cholesterol-free, very-low-fat diet, it did increase (or at least restore) receptor expression that had been downregulated by dietary cholesterol and triglyceride. Thus, 7.5% dietary psyllium produced effects on hepatic 7α-hydroxylase and LDL metabolism that were similar to those of 1% cholestyramine. Induction of hepatic 7α-hydroxylase activity by dietary psyllium may account, in large part, for the hypocholesterolemic effect of this soluble fiber. (*J. Clin. Invest.* 1994. 2084–2092.) Key words: dietary fiber • 7α-hydroxylase • LDL transport • liver • cholesterol • bile acids

Introduction

Western populations typically consume diets high in fat and low in fiber. These diets have been linked epidemiologically to a number of diseases common to modern societies including coronary heart disease, cholesterol gallstone disease, and colon cancer (1–6). The role of individual dietary components in the initiation and promotion of these disease processes and the mechanistic basis of their effects remain poorly understood. Dietary fiber has important physiologic effects on large bowel function and certain types of fiber are known to lower plasma cholesterol concentrations in experimental animals and humans (1, 2). Psyllium is a hydrophilic, gel-forming polymer that is currently in widespread use in the management of diverticular disease and functional disorders of the large bowel. In addition, psyllium has important effects on cholesterol and lipoprotein metabolism and has been shown to lower plasma LDL cholesterol concentrations in experimental animals and humans (7–12). How soluble fibers lower plasma cholesterol concentrations remains incompletely defined although numerous hypotheses have been proposed, including binding or sequestration of bile acids by fiber leading to an increase in fecal bile acid excretion, disruption of micelle formation leading to lipid malabsorption, and suppression of hepatic sterol synthesis by fermentation products of dietary fiber (2). None of these hypotheses is entirely consistent with all of the data currently available, suggesting that multiple mechanisms may contribute to the hypocholesterolemic effects of soluble fibers and/or that the principal mechanism of action may differ among various soluble fibers.

Conversion of cholesterol to bile acids represents the major regulated pathway whereby cholesterol is eliminated from the body (13, 14). Hepatic 7α-hydroxylase is the initial and rate-limiting enzyme in the bile acid biosynthetic pathway. The precise mechanisms involved in the regulation of 7α-hydroxylase are not entirely clear; however, the enzyme appears to be subject to feedback inhibition by bile acids fluxing through the liver in the enterohepatic circulation (14, 15). Cholestyramine is an anion exchange resin that avidly binds bile acids in the intestinal lumen thereby preventing their reabsorption. Loss of bile acids from the enterohepatic circulation results in derepression of 7α-hydroxylase activity and an increase in the rate of bile acid synthesis. The liver compensates for the loss of cholesterol by increasing the rate of de novo cholesterol synthesis and, in many cases, by up-regulating hepatic LDL receptor activity (16–18). Since the liver is the major site of LDL catabolism, and since LDL uptake by the liver is mediated largely by LDL receptors, changes in hepatic LDL receptor activity generally effect reciprocal changes in circulating LDL levels.

A similar mechanism of action has been proposed to explain the hypocholesterolemic effects of psyllium (1, 2). However, unlike cholestyramine, psyllium displays little if any bile acid binding activity in vitro (7). Moreover, in most studies psyllium induces only a marginal (19–21), or no (22), increase in the turnover and excretion of bile acids, especially when compared with the effects of cholestyramine (19, 23). Since the evidence supporting other proposed mechanisms is even less compelling, attention is again being focused on the hypothesis that soluble fiber may lower plasma cholesterol levels via effects on bile acid metabolism (21, 24, 25).

The present studies were undertaken to determine the effect of dietary psyllium on the expression of 7α-hydroxylase,

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3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA)[1] reductase and the LDL receptor and to compare the effects of psyllium to those of a known bile acid sequestrant, cholestyramine. These studies show that dietary psyllium up-regulates hepatic 7α-hydroxylase expression—an effect that may account for the known hypocholesterolemic activity of this soluble fiber.

Methods

Animals and diets. Male Golden Syrian hamsters (Sasco, Inc., Omaha, NE) were housed in colony cages and subjected to light cycling for at least 3 wk before introduction of the experimental diets. The control semisynthetic diet used in most studies contained (wt/wt) 20% soy protein, 60.5% corn starch, 2% corn oil, 0.3% DL-methionine, 8.5% salt mix, 1% vitamin mix, 0.2% choline bitartrate, and 7.5% cellulose (~ 22 g/1,000 kcal). The experimental diets were prepared by replacing the cellulose with psyllium (kindly provided by Dr. Bruce Daggy, The Procter & Gamble Company, Cincinnati, OH) or cholestyramine on a wt/wt basis. A preliminary dose–response study was performed using the control diet described above except that the fiber content was increased to 10% by weight. In some studies, the effects of psyllium were examined in animals fed a diet enriched with cholesterol and triglyceride. This diet was prepared by isocalorically replacing corn starch in the semisynthetic control diet with coconut oil and safflower oil to provide 24 and 12% of total calories, respectively, and including cholesterol at the 0.08% level (~ 190 mg/1,000 kcal). The avicel content of the diet was maintained at 7.5% by wt (~ 18 g/1,000 kcal) and the experimental diets were prepared by replacing avicel with psyllium or cholestyramine on a wt/wt basis. The various diets were fed ad lib for 1 mo and all studies were carried out during the mid-dark phase of the light cycle unless otherwise indicated.

Determination of hepatic 7α-hydroxylase activity. Hepatic 7α-hydroxylase activity was measured using an HPLC-spectrophotometric assay that quantifies the mass of 7α-hydroxycholesterol formed from endogenous microsomal cholesterol after enzymatic conversion to 7α-hydroxy-4-cholesten-3-one using cholesterol oxidase (26).

Determination of hepatic cholesterol synthesis rates. Rates of hepatic cholesterol synthesis were measured in vivo using [^{3}H]water. As previously described (27), the animals were administered ~ 100 mCi of [^{3}H]water intravenously and then returned to individual cages under a fume hood. 1 h after the injection of [^{3}H]water, 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 were taken for the isolation of digitonin-precipitable sterols. Rates of sterol synthesis are expressed as the nmoles of [^{3}H]water incorporated into digitonin-precipitable sterols per hour per gram of liver (nmoles/h/g).

Determination of hepatic LDL uptake rates in vivo. Plasma was obtained from normocholesterolemic hamster and human donors. The LDL was isolated from plasma by preparative ultracentrifugation in the density range of 1.020 to 1.055 g/ml and labeled with ^{125}I- or ^{131}I-tyramine cellobiose as previously described (28). The human LDL was also reductively methylated to completely eliminate its recognition by the LDL receptor (29). Rates of hepatic LDL uptake were measured using primed-infusions of ^{125}I-tyramine cellobiose-labeled LDL. The infusions of ^{125}I-tyramine cellobiose-labeled LDL were continued for 4 h at which time each animal was administered a bolus of ^{131}I-tyramine cellobiose-labeled LDL as a volume marker and killed 10 min later by exsanguination through the abdominal aorta. Tissue samples along with aliquots of plasma were assayed for radioactivity in a gamma counter (Packard Instrument Co., Inc., Downers Grove, IL). The amount of labeled LDL in the various tissues at 10 min (^{131}I disintegrations per minute per gram of tissue divided by the specific activity of ^{131}I in plasma) and at 4 h (^{125}I disintegrations per minute per gram of tissue divided by the specific activity of ^{125}I in plasma) was then calculated. The increase in the tissue content of LDL cholesterol or LDL protein with time represents the rate of LDL uptake in micrograms of LDL cholesterol or LDL protein taken up per hour per gram of tissue.

Since receptor-dependent LDL uptake by the liver is saturable, and since plasma LDL concentrations varied considerably among the different experimental groups, changes in receptor-dependent LDL uptake could not be directly equated with changes in LDL receptor activity (30). To relate changes in receptor-dependent LDL uptake to changes in LDL receptor activity, the experimentally determined uptake rates were superimposed on kinetic curves describing the relationship between hepatic LDL uptake and circulating LDL concentrations in normal animals. By relating the rates of receptor-dependent and receptor-independent LDL uptake in the experimental animals to these normal kinetic curves, it was possible to determine how the various dietary manipulations affected LDL receptor activity (defined as the rate of receptor-dependent LDL uptake in an experimental animal relative to the rate of receptor-dependent LDL uptake seen in control animals at the same plasma LDL concentration).

Determination of mRNA levels. Hepatic 7α-hydroxylase, HMG CoA reductase, LDL receptor, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, used as an invariant control) mRNA levels were determined by nuclease protection as previously described (31). A cDNA probe was not available for Syrian hamster 7α-hydroxylase. Therefore, following reverse transcriptase synthesis of the complementary DNA, the polymerase chain reaction (PCR) was used to amplify a fragment of the 7α-hydroxylase cDNA from Syrian hamster liver RNA. Oligonucleotide primers used to amplify a 241 nt 7α-hydroxylase cDNA fragment (5′-AAAGGATCCCTACTTCTGCGAAGGCATTTGG-3′, and 5′-AAAGAATTCCCAGCTTCAAACATCACTCGGTA-3′) were selected from areas of 100% homology in the published rat and human 7α-hydroxylase sequences (32, 33). The PCR was carried out sequentially for 5 min at 55°C, 2 min at 72°C and 45 s at 95°C for 30 cycles in a programmable thermal controller (MJ Research, Inc., Watertown, MA). The oligonucleotide primers were synthesized with restriction sites (BamHI and EcoRI) to allow direct subcloning of the amplified DNA into the plasmid pGEM (Promega Corp., Madison, WI) for sequencing and into the bacteriophage M13 (Pharmacia LKB Biotech, Piscataway, NJ) for the preparation of ^{32}P-labeled single-stranded probes. The cDNA probes for Syrian hamster LDL receptor (402 nt) and GAPDH (204 nt) were previously described (31). A cDNA for the Syrian hamster HMG CoA reductase (pDGS6) was obtained from Robert Simoni (University of California, Berkley, CA) and a 393 nt HindIII fragment subcloned into M13. Probes were synthesized as previously described (31) using 0.5 μM [^{32}P]dCTP and 1 μM (7α-hydroxylase, HMG CoA reductase and LDL receptor) or 300 μM (GAPDH) unlabeled dCTP.

Samples of hamster liver were homogenized in guanidinium thiocyanate and the RNA isolated by the method of Chomczynski and Sacchi (34). 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 U of mung bean nuclease (GIBCO BRL [Life Technologies, Inc.] Gaithersburg, MD). The mRNA-protected ^{32}P-labeled probes were separated on 7 M urea, 6% polyacrylamide gels together with ^{32}P-labeled MspI-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). The level of GAPDH mRNA did not vary among the various experimental groups and was used to correct for any procedural losses.

Determination of liver and plasma cholesterol distribution. Liver cholesterol was quantified by capillary gas–liquid chromatography. The cholesterol distribution in plasma was determined by gel filtration chromatography using a superose 6 column (Sigma Chemical Co., St. Louis, MO). 2-ml aliquots were collected and assayed for cholesterol

1. *Abbreviations used in this paper:* GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A.

using an enzymatic kit (Boehringer Diagnostics, Indianapolis, IN).

Statistical analysis. The data are presented as means±1 SD. To test for differences among the dietary regimens, one-way analysis of variance was performed. Significant results were further analyzed using the Tukey multiple comparison procedure.

Results

Experiments were first undertaken to determine the effect of dietary psyllium on the expression of hepatic 7α-hydroxylase, the initial and rate-limiting enzyme in the bile acid biosynthetic pathway. In preliminary studies, a dose–response experiment to determine the optimal dose of psyllium for further study was performed and the results are shown in Fig. 1. The cholesterol-free semisynthetic control diet used in this study contained 10% by wt microcrystalline cellulose (avicel). Avicel is a water insoluble fiber that appears to have little if any effect on sterol metabolism and is added to semisynthetic diets at the 6-10% level to allow normal bowel function. In the experimental diets, avicel was replaced with the hydrophilic, gel-forming fiber psyllium. As shown in Fig. 1, 7α-hydroxylase activity equaled 59±15 pmol/h per mg microsomal protein in animals fed the control diet and increased 1.3-, 2.0-, 3.1-, and 3.4-fold in animals fed psyllium at the 2.5, 5, 7.5, and 10% levels, respectively. Plasma cholesterol levels were measured in the same animals and fell by 14, 21, 26, and 46% when psyllium was fed at the 2.5, 5, 7.5, and 10% levels, respectively. Weight gain was similar in all groups with the exception of the 10% psyllium group which gained significantly less weight than the control group. Subsequent studies were therefore performed using semisynthetic diets containing 7.5% (by weight) avicel or psyllium. For comparative purposes, diets were also fed in which avicel was partially replaced with the bile acid sequestrant cholestyramine. In most studies, cholestyramine was fed at the 1% level since preliminary studies showed this dose of cholestyramine to be about as active as 7.5% psyllium in increasing 7α-hydroxylase activity.

Fig. 2 shows the mean changes in hepatic 7α-hydroxylase activity and mRNA in hamsters fed the psyllium or cholestyramine diets for 1 mo. As shown in the top panel, hepatic 7α-hydroxylase activity increased about fourfold when avicel was completely replaced by psyllium in the diet. By comparison,

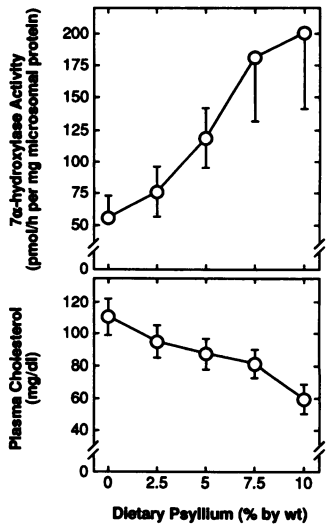

Figure 1. Dose–response relationship between hepatic 7α-hydroxylase activity and the psyllium content of the diet. Animals were fed a cholesterol-free, very-low-fat semisynthetic diet containing 10% avicel, or experimental diets in which avicel was replaced by psyllium on a wt/wt basis. 7α-hydroxylase activity was measured using endogenous microsomal cholesterol as substrate. Each value represents the mean±1 SD for data obtained in 12 animals.

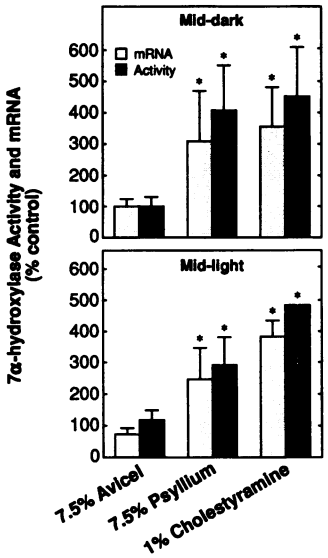

Figure 2. Regulation of hepatic 7α-hydroxylase activity and mRNA by dietary psyllium. Animals were adapted to normal or reverse-phase light cycling and fed a cholesterol-free, very-low-fat semisynthetic diet containing 7.5% avicel, or experimental diets in which avicel was replaced by psyllium or cholestyramine on a wt/wt basis. The top and bottom panels show data obtained at the mid-dark and mid-light points of the light cycle, respectively. 7α-hydroxylase activity was measured using endogenous microsomal cholesterol as substrate. 7α-hydroxylase activity equaled 66±14 pmol/h per milligram of microsomal protein in the control mid-dark group and activities in the experimental groups are expressed as a percentage of this value. Each value represents the mean±1 SD for data obtained in 12 (mid-dark) or 6 (mid-light) animals. *Significantly differs from the corresponding avicel group, $P < 0.05$.

hepatic 7α-hydroxylase activity increased ~ 4.5-fold in animals fed 1% cholestyramine. Samples of liver were taken from these same animals for the determination of 7α-hydroxylase mRNA levels. 7α-hydroxylase mRNA was quantified by nuclease protection using single-stranded cDNA probes specific for the Syrian hamster. An autoradiogram depicting the effect of dietary psyllium and cholestyramine on hepatic 7α-hydroxylase mRNA is shown in Fig. 3 and the mean changes in 7α-hydroxylase mRNA are summarized in Fig. 2. As shown in the top panel of Fig. 2, hepatic 7α-hydroxylase mRNA increased by ~ 3-fold in animals fed psyllium and by ~ 3.5-fold in animals fed 1% cholestyramine. Overall, the changes in 7α-hydroxylase activity in animals fed the psyllium and cholestyramine diets could be accounted for largely by changes in 7α-hydroxylase mRNA levels. Thus, the replacement of dietary avicel with psyllium resulted in a significant increase in hepatic 7α-hydroxylase expression that was similar in magnitude to that seen with modest doses of bile acid sequestrants.

Since the half-life of 7α-hydroxylase is relatively short, it is possible that measurements made at a single time point during the 24-h light cycle may not reflect conditions throughout the

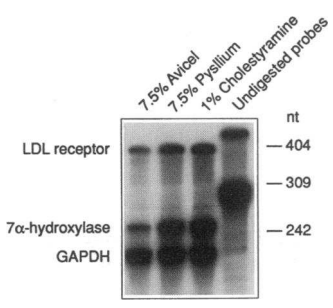

Figure 3. Measurement of hepatic 7α-hydroxylase mRNA levels. Hepatic RNA was isolated from animals fed the diets described in the legend to Fig. 2. Total RNA (40 μg) was hybridized with ^{32}P-labeled single-stranded cDNA probes and the protected bands resistant to mung bean nuclease digestion were analyzed by polyacrylamide gel electrophoresis followed by autoradiography.

remainder of the light cycle. Moreover, in some species such as the rat, the activity of 7α-hydroxylase exhibits marked diurnal fluctuations with activity during the mid-dark phase of the light cycle being several-fold higher than during the mid-light phase (35). To determine if hamster 7α-hydroxylase is subject to diurnal variation and whether the stimulatory effects of dietary psyllium are manifest at other time points in the light cycle, studies were performed at the mid-light timepoint in animals adapted to reverse light cycling (Fig. 2, *bottom panel*). The level of 7α-hydroxylase expression in control animals was similar at the mid-light and mid-dark time points. Dietary psyllium and cholestyramine significantly increased hepatic 7α-hydroxylase activity and mRNA at both timepoints although the stimulatory effect of psyllium was somewhat less during the mid-light than during the mid-dark phase of the light cycle.

The major form of regulation of 7α-hydroxylase expression appears to be feedback inhibition of gene transcription by hydrophobic bile acids in the enterohepatic circulation. Thus, reducing the enterohepatic pool of bile acids by administering bile acid sequestrants or by biliary diversion results in derepression of 7α-hydroxylase expression whereas expanding the enterohepatic pool by administering bile acids suppresses 7α-hydroxylase expression. If psyllium acts by interfering with the absorption or enterohepatic cycling of bile acids, then it should be able to counteract the suppressive effects of bile acids on 7α-hydroxylase expression. We therefore examined the effect of dietary psyllium on 7α-hydroxylase expression in animals fed 0.1% cholic acid and these data are shown in Fig. 4. When added to the avicel diet, cholic acid markedly suppressed hepatic 7α-hydroxylase activity and mRNA—an effect that was largely prevented by dietary psyllium. Indeed, when added to the cholic acid–containing diet, psyllium increased 7α-hydroxylase activity by ~ 25-fold—to levels ~ 2.5-fold higher than those of animals fed avicel alone. As also shown in Fig. 4, the ability of psyllium to counteract the inhibitory effect of cholic acid was similar to that of 1% cholestyramine. How psyllium might alter the absorption or enterohepatic cycling of bile acids is unclear since, unlike cholestyramine, psyllium exhibits no bile acid binding activity in vitro (7).

In response to changes in net sterol input or output, the liver maintains cholesterol homeostasis by adjusting the rate of de novo cholesterol synthesis and, in some cases, by regulating the LDL receptor pathway (13, 36). Fig. 5 shows the changes in hepatic cholesterol synthesis in animals fed the psyllium or cholestyramine diets for 4 wk. Rates of hepatic cholesterol synthesis equaled 120±42 nmol/h per gram in animals fed avicel and increased 5.1-fold when avicel was replaced with psyllium. By comparison, hepatic sterol synthesis increased 8.6-fold in animals fed 1% cholestyramine. As also shown in Fig. 5, hepatic mRNA levels for HMG CoA reductase increased 1.8- and 2.7-fold in animals fed psyllium or 1% cholestyramine, respectively. These changes in HMG CoA reductase mRNA levels were much smaller than the corresponding changes in rates of cholesterol synthesis suggesting that posttranscriptional regulation of HMG CoA reductase activity contributed significantly to the changes in cholesterol synthesis.

The studies described above investigating the effect of dietary psyllium on hepatic sterol metabolism were carried out in the context of a cholesterol-free, very-low-fat diet. To examine the effect of dietary psyllium under dietary conditions more

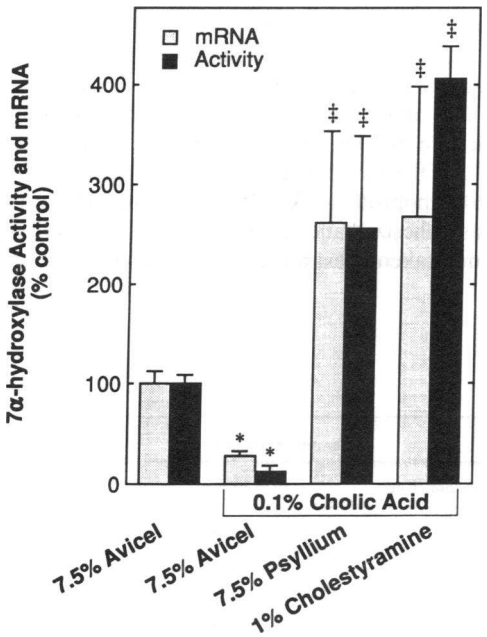

Figure 4. Effect of dietary psyllium on hepatic 7α-hydroxylase expression during expansion of the enterohepatic pool of bile acids. Animals were fed a cholesterol-free, very-low-fat semisynthetic diet containing 7.5% avicel and 0.1% cholic acid or the same diet in which avicel was replaced by psyllium or cholestyramine on a wt/wt basis. Each value represents the mean±1 SD for data obtained in 6 animals. *Significantly differs from the corresponding avicel group fed no cholic acid, $P < 0.05$. ‡Significantly differs from the corresponding avicel groups, $P < 0.05$.

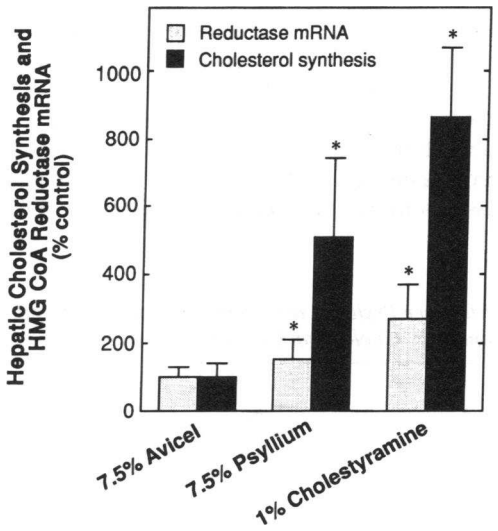

Figure 5. Regulation of hepatic cholesterol synthesis and HMG CoA reductase mRNA by dietary psyllium. Animals were fed a cholesterol-free, very-low-fat semisynthetic diet containing 7.5% avicel, or experimental diets in which avicel was replaced by psyllium or cholestyramine on a wt/wt basis. Rates of hepatic cholesterol synthesis were quantified in vivo using [^{3}H]water. The rate of cholesterol synthesis equaled 120±42 nmol/h per gram of liver in the control group and synthesis rates in the experimental groups are expressed as a percentage of this value. Each value represents the mean±1 SD for data obtained in 12 animals. *Significantly differs from the corresponding avicel group, $P < 0.05$.

relevant to western humans, studies were carried out using diets enriched with cholesterol and triglyceride. For these studies, animals were fed a semisynthetic diet containing 0.08% cholesterol (~ 190 mg/1,000 kcal), 24% energy coconut oil and 12% energy safflower oil. Avicel was present at the 7.5% by wt level (~ 18 g/1,000 kcal) and the experimental diets were prepared by replacing avicel with psyllium or cholestyramine on a wt/wt basis. Fig. 6 shows the effect of dietary psyllium on hepatic 7α-hydroxylase activity and mRNA in animals fed diets enriched with cholesterol and saturated fatty acids. Hepatic 7α-hydroxylase activity equaled 51±13 pmol/h per milligram of microsomal protein in the avicel-containing diet and increased 5.1-fold when avicel was completely replaced with psyllium. By comparison, hepatic 7α-hydroxylase activity increased 5.9-fold in animals fed 1% cholestyramine. As also shown in Fig. 6, the changes in 7α-hydroxylase activity in animals fed the psyllium and cholestyramine diets could be accounted for largely by changes in 7α-hydroxylase mRNA levels.

Thus, compared with avicel, psyllium increased hepatic 7α-hydroxylase expression severalfold not only in animals fed a cholesterol-free, very-low-fat diet but also in animals fed a diet enriched in cholesterol and triglyceride whose composition more closely resembles that of western diets. As noted above, the resulting net loss of sterol from the liver triggers an increase in the rate of de novo cholesterol synthesis; however, this increase in synthesis is not fully compensatory since the cholesterol content of the liver significantly fell in animals fed psyllium or cholestyramine, as shown in Table I. In animals fed the cholesterol-free, very-low-fat diet, the cholesterol content of the liver fell from 2.4 mg/g to 2.0 mg/g when avicel was completely replaced with psyllium and to 1.9 mg/g with 1% cholestyramine. Changes in liver cholesterol were much greater in animals fed the diet enriched with cholesterol and triglyceride, where the cholesterol content of the liver fell from 15.2 mg/g to 2.4 mg/g when avicel was completely replaced with psyllium and to 2.1 mg/g with 1% cholestyramine.

Like HMG CoA reductase, the LDL receptor pathway is subject to feedback inhibition by cellular sterols and changes in cellular cholesterol balance generally result in coordinate regulation of these two pathways. As much as 80% of total LDL turnover is mediated by LDL receptors (37), the vast majority of which are located in the liver (30). Thus changes in hepatic LDL receptor activity may profoundly alter circulating LDL concentrations. The other major determinant of circulating LDL levels is the rate of LDL entry into plasma (total LDL transport or flux). LDL arise in plasma during the metabolism of very low density lipoproteins (VLDL), which are triglyceride-rich particles synthesized and secreted by the liver. Studies were therefore undertaken to examine the effect of psyllium on

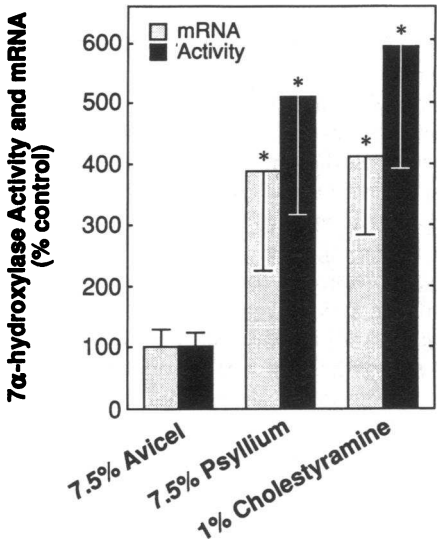

Figure 6. Regulation of hepatic 7α-hydroxylase activity and mRNA by dietary psyllium in animals fed diets enriched with cholesterol and triglyceride. Animals were fed a semisynthetic diet enriched with cholesterol (0.08% by weight) and triglyceride (coconut oil and safflower oil to provide 24% energy and 12% energy, respectively). The diet contained 7.5% avicel and experimental diets were prepared by replacing avicel with psyllium or cholestyramine on a wt/wt basis. 7α-hydroxylase activity equaled 51±13 pmol/h per milligram of microsomal protein in the avicel group and activities in the experimental groups are expressed as a percentage of this value. Each value represents the mean±1 SD for data obtained in six animals. *Significantly differs from the corresponding avicel group, $P < 0.05$.

Table I. Effect of Dietary Psyllium and Cholestyramine on Hepatic Cholesterol Content and Plasma Lipoprotein–Cholesterol Concentrations

Diet	Liver weight	Liver cholesterol	Plasma cholesterol		
			VLDL + chylomicrons	LDL	HDL
	g/100 g body wt	*mg/g*		*mg/dl*	
Low fat					
7.5% Avicel	3.5±0.3	2.4±0.2	6±1	28±3	69±8
7.5% Psyllium	3.1±0.3*	2.0±0.1*	5±1	22±3*	57±7*
1% Cholestyramine	3.1±0.2*	1.9±0.1*	5±1	21±3*	61±8
High fat					
7.5% Avicel	4.3±0.5	15.2±4	38±6	64±7	102±13
7.5% Psyllium	3.2±0.3*	2.4±0.3*	10±2*	33±4*	65±8*
1% Cholestyramine	3.3±0.3*	2.1±0.5*	9±3*	27±5*	69±9*

Each value represents the mean±1 SD for data obtained in 6–12 animals. * Significantly different from the corresponding avicel group at $P < 0.05$.

plasma LDL levels and on the two major transport processes that control the concentration of this lipoprotein in plasma, i.e., the rate of receptor-dependent LDL uptake by the liver and the rate of LDL entry into plasma.

The effect of psyllium and cholestyramine on plasma LDL concentrations is shown in Table I. In animals fed the cholesterol-free, very-low-fat diet, plasma LDL cholesterol concentrations fell from 28 mg/dl to 22 mg/dl when avicel was completely replaced with psyllium and to 21 mg/dl in animals fed 1% cholestyramine. As with liver cholesterol, changes in plasma LDL cholesterol concentrations were more substantial in animals fed the diet enriched with cholesterol and triglyceride, where LDL cholesterol fell from 64 mg/dl to 33 mg/dl when avicel was completely replaced with psyllium and to 27 mg/dl in animals fed 1% cholestyramine. HDL cholesterol levels also fell in animals fed the psyllium and cholestyramine diets, although the percentage reductions in this lipoprotein fraction were somewhat less than those seen in the LDL fraction (Table I).

Absolute rates of total and receptor-independent LDL uptake by the various tissues of the body were measured in vivo using hamster and methylated human LDL, respectively. Receptor-dependent LDL uptake was taken as the difference between total and receptor-independent uptake. To quantify changes in hepatic LDL receptor activity in vivo, absolute rates of LDL uptake in the experimental animals were related to standard kinetic curves describing the relationship between LDL uptake and circulating LDL concentrations in control animals. Fig. 7 shows the kinetic curves for normal hepatic LDL transport in the hamster. The shaded areas represent the relationship between total (*stippled*) and receptor-independent (*hatched*) LDL uptake and plasma LDL concentrations over the range of LDL concentrations observed in these studies.

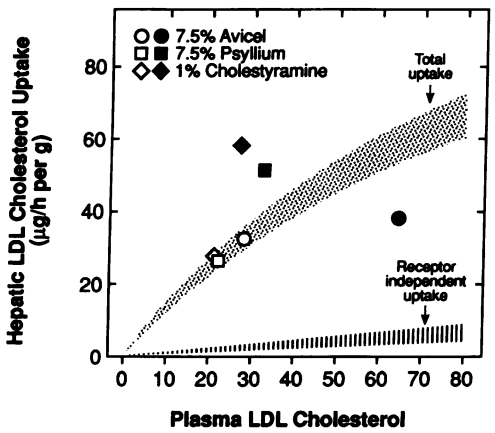

Figure 7. Effect of dietary psyllium on hepatic LDL uptake. Animals were fed a cholesterol-free, very-low-fat semisynthetic diet (*open symbols*) or a semisynthetic diet enriched with cholesterol (0.08% by weight) and triglyceride (coconut oil and safflower oil to provide 24% energy and 12% energy, respectively). Both diets contained 7.5% avicel and experimental diets were prepared by replacing avicel with psyllium or cholestyramine on a wt/wt basis. The shaded areas represent the kinetic curves for total (*stippled*) and receptor-independent (*hatched*) LDL cholesterol uptake determined in control animals as described in Methods. Superimposed on these normal kinetic curves are the absolute rates of total LDL cholesterol uptake in the experimental animals plotted as a function of the plasma LDL cholesterol concentration in the same animals.

These standard kinetic curves were previously established by quantifying rates of total and receptor-independent LDL uptake in control animals under conditions where plasma LDL concentrations were acutely raised and maintained at various levels by infusions of unlabeled LDL (30). The mean values for total LDL transport in animals fed the psyllium and cholestyramine diets are superimposed on these standard kinetic curves. Rates of receptor-independent LDL transport were normal in all experimental groups and are not shown. In animals fed the cholesterol-free, very-low-fat diet, rates of total (*open circles*) and receptor-independent LDL cholesterol uptake fell on the standard kinetic curves for LDL transport in normal animals and receptor activity in these animals was assigned a value of 100%. Similarly, when avicel was replaced by psyllium (*open square*) or cholestyramine (*open diamond*), rates of total and receptor-independent LDL cholesterol uptake were not significantly displaced from the standard kinetic curves, indicating no change in the receptor-dependent or receptor-independent pathways.

In animals fed the diet enriched with cholesterol and triglyceride, the rate of total LDL cholesterol uptake equaled 39 μg/h per gram at a plasma LDL cholesterol concentration of 64 mg/dl (*solid circle*), whereas normal animals transport ~ 59 μg/h per g at this LDL concentration. Since receptor-independent LDL transport was normal in these animals, the decrease in total LDL cholesterol uptake could be attributed entirely to a decrease in receptor-dependent LDL uptake. In contrast, when avicel was replaced by psyllium (*solid square*) or cholestyramine (*solid diamond*) in animals fed the high fat diet, rates of total LDL cholesterol uptake exceeded those observed in normal animals at the same plasma LDL concentration. Again, since receptor-independent LDL transport was normal, the increase in total LDL cholesterol uptake could be attributed entirely to an increase in receptor-dependent LDL uptake. From this type of analysis, changes in hepatic LDL receptor activity (defined as the rate of receptor-dependent LDL uptake in experimental animals relative to the rate of receptor-dependent LDL uptake in control animals at the same plasma LDL cholesterol concentration) were calculated for each of the experimental groups. In addition, LDL uptake rates in the various extrahepatic tissues were measured and the sum of uptake rates in all of the tissues of the body was taken as a measure of total LDL transport, which in a steady-state, must equal the rate of LDL formation.

The effects of dietary psyllium on hepatic LDL receptor expression and rates of LDL formation in animals fed the cholesterol-free, very-low-fat diet are summarized in Fig. 8. As shown in the top panel, replacement of avicel with psyllium had no significant effect on hepatic LDL receptor activity or mRNA. Similarly, 1% cholestyramine had no significant effect on hepatic LDL receptor activity or mRNA although doses of 2% or more significantly upregulated LDL receptor expression (data not shown). As shown in the bottom panel, total LDL cholesterol transport in animals fed the cholesterol-free, very-low-fat control diet equaled 151±23 μg/h per 100 g body weight. Replacement of avicel with psyllium or 1% cholestyramine significantly reduced total LDL cholesterol transport (by 28 and 18%, respectively) and these reductions in the rate of LDL formation accounted entirely for the decline in plasma LDL cholesterol concentrations observed in these animals.

The effects of dietary psyllium on hepatic LDL receptor expression and rates of LDL formation in animals fed diets

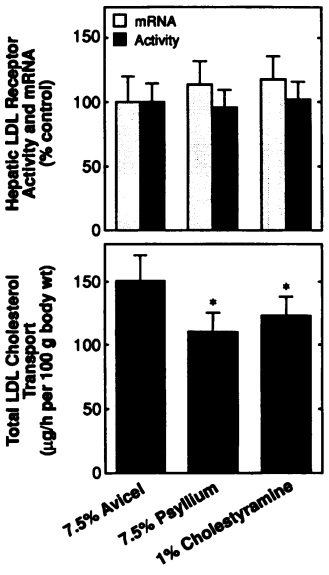

Figure 8. Effect of dietary psyllium on hepatic LDL receptor expression and total LDL cholesterol transport in animals fed a low fat diet. Animals were fed a cholesterol-free, very-low-fat semisynthetic diet containing 7.5% avicel or experimental diets in which avicel was replaced by psyllium or cholestyramine on a wt/wt basis. Changes in whole liver LDL receptor activity were determined as illustrated in Fig. 7, taking into account the changes in liver weight shown in Table I. Each value represents the mean±1 SD for data obtained in 12 animals. *Significantly differs from the corresponding avicel group, $P < 0.05$.

enriched with cholesterol and triglyceride are summarized in Fig. 9. The level of hepatic LDL receptor expression was lower and the rate of LDL formation higher in animals fed the diet enriched with cholesterol and triglyceride compared to animals fed the cholesterol-free, very-low-fat control diet. Under these conditions, replacement of avicel with psyllium or 1% cholestyramine significantly increased (or restored) hepatic LDL receptor activity and mRNA. Taking into account changes in liver size (Table I), whole liver LDL receptor activity in the psyllium and cholestyramine groups was 64 and 136% higher, respectively, than in the avicel group. As shown in the bottom panel, total LDL cholesterol transport in animals fed the diet enriched with cholesterol and triglyceride equaled 257±41 µg/h per 100 g body wt. Replacement of avicel with psyllium or 1% cholestyramine significantly reduced total LDL cholesterol transport by 26 and 19%, respectively.

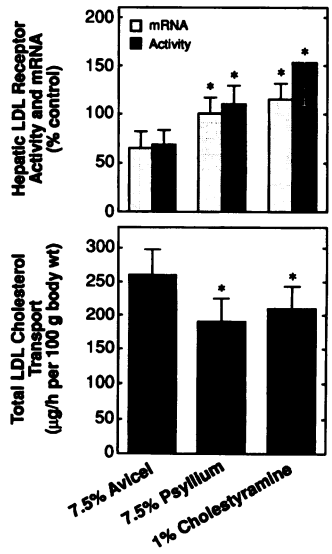

Figure 9. Effect of dietary psyllium on LDL receptor expression and total LDL cholesterol transport in animals fed diets enriched with cholesterol and triglyceride. Animals were fed a semisynthetic diet enriched with cholesterol (0.08% by weight) and triglyceride (coconut oil and safflower oil to provide 24% energy and 12% energy, respectively). The diet contained 7.5% avicel and experimental diets were prepared by replacing avicel with psyllium or cholestyramine on a wt/wt basis. Changes in whole liver LDL receptor activity were determined as illustrated in Fig. 7, taking into account the changes in liver weight shown in Table I. Each value represents the mean±1 SD for data obtained in 12 animals. *Significantly differs from the corresponding avicel group, $P < 0.05$.

Discussion

Hepatic 7α-hydroxylase is the initial and rate-limiting enzyme in the pathway that converts cholesterol to bile acids. Although the molecular mechanisms involved in the regulation of 7α-hydroxylase gene expression are not fully understood, the enzyme does appear to be subject to feedback inhibition by bile acids returning to the liver in the enterohepatic circulation (14, 15, 38, 39). Thus, the oral administration of cholestyramine—an anion exchange resin that avidly binds bile acids in vitro and in vivo—results in loss of bile acids from the enterohepatic circulation and derepression of hepatic 7α-hydroxylase gene expression. In the present studies, hepatic 7α-hydroxylase activity and mRNA levels increased by 3–4-fold in hamsters when avicel (the inert water-insoluble fiber generally added to semisynthetic diets) was replaced by psyllium (a water-soluble gel-forming fiber). This increase in hepatic 7α-hydroxylase expression was similar in magnitude to that observed with a modest dose (1%) of cholestyramine.

If psyllium increases 7α-hydroxylase expression by interfering with the enterohepatic cycling of bile acids, this effect should be exaggerated under conditions where the enterohepatic pool of bile acids has been expanded. As expected, cholic acid markedly suppressed hepatic 7α-hydroxylase activity and mRNA levels when added to the control semisynthetic diet in amounts known to increase the total bile acid pool by ~ 50% (40). Under these conditions, replacement of the avicel with psyllium (or partial replacement with cholestyramine) led to derepression of hepatic 7α-hydroxylase activity and mRNA, to levels 2–3-fold higher than in the control animals ingesting no bile acid. Together, these data suggest that dietary psyllium alters the absorption or enterohepatic cycling of bile acids thereby resulting in derepression of hepatic 7α-hydroxylase expression. The mechanism of this effect is unclear since psyllium exhibits no bile acid binding activity in vitro (7). It is possible that the hydrated psyllium gel nonspecifically traps or alters the diffusion of bile acids in the gut resulting in increased fecal losses. Neutral steroid excretion is consistently unaffected by dietary psyllium, however, casting some doubt on this mechanism (8, 20, 24).

Although dietary psyllium increased 7α-hydroxylase expression by 3–4-fold, it was much less active in this regard than cholestyramine when expressed on a g/g basis. Thus, 7.5% psyllium induced 7α-hydroxylase expression somewhat less effectively than 1% cholestyramine. Most previous studies dealing with the mechanism of psyllium's hypocholesterolemic effect have shown a marginal increase in bile acid turnover or fecal bile acid excretion (19–21, 24). Taken together, the data suggest that psyllium is approximately one tenth as active as cholestyramine (on a g/g basis) in increasing fecal bile acid excretion and stimulating hepatic 7α-hydroxylase. Although not highly effective, soluble fiber is widely distributed in many foods. Fiber intake varies by 5–10-fold among different populations and it is likely that this factor contributes, at least in part, to the observed cross-sectional differences in plasma cholesterol concentrations.

In a steady state, the amount of cholesterol entering the body by way of the diet plus the amount of cholesterol synthesized de novo in the body must equal the amount of cholesterol that is eliminated from the body. The liver plays a key role in the maintenance of whole body sterol balance in that the liver is the principal destination for cholesterol absorbed from the

gut, and secretion of cholesterol into bile, either as such or after conversion to bile acids, represents the only significant route for cholesterol elimination from the body. In response to changes in cholesterol input or loss, the liver compensates by adjusting the rate of de novo cholesterol synthesis. In addition, the liver may alter the influx of lipoprotein cholesterol (by regulating the LDL receptor pathway) or alter the amount of lipoprotein cholesterol secreted into plasma. These latter adaptive responses of the liver to changes in whole body sterol balance can lead to marked alterations in circulating LDL levels since the liver is the sole source of LDL (via VLDL) and is the major site of LDL catabolism.

In the present studies, psyllium increased hepatic 7α-hydroxylase activity and mRNA by 3–4-fold, which, in turn, led to a compensatory fivefold increase in hepatic cholesterol synthesis. Although it has been suggested that the hypocholesterolemic effect of psyllium is due to suppression of hepatic sterol synthesis by propionic acid and other products generated during the fermentation of psyllium (2), recent studies in the rat and hamster have clearly demonstrated derepression of hepatic sterol synthesis (7, 41) and it appears that the suppression of hepatic sterol synthesis observed in earlier studies may have been a technical artifact related to dilution of the radiolabeled acetate precursor by unlabeled short chain fatty acids. To the extent that data are available, high fiber diets also appear to increase endogenous sterol synthesis in humans (42).

In addition to derepression of hepatic sterol synthesis, replacement of avicel with psyllium significantly lowered hepatic and plasma cholesterol concentrations. In animals fed the cholesterol-free, very-low-fat diet, the decline in plasma LDL cholesterol levels was quite modest (from 26 to 22 mg/dl or 21%)—similar to that observed with 1% cholestyramine. These relatively small changes in plasma LDL concentrations were not due to changes in receptor-dependent LDL uptake by the liver but rather to a reduction in the rate of LDL formation. Thus, under these conditions, loss of cholesterol from the liver induced by psyllium or 1% cholestyramine was associated with a several-fold increase in hepatic sterol synthesis but no change in hepatic LDL receptor expression. Higher doses of cholestyramine, however, clearly induced the LDL receptor pathway suggesting a hierarchical pattern of regulation of HMG CoA reductase and the LDL receptor pathway. The recent isolation and purification of nuclear proteins that are unique for the transcriptional regulation of HMG CoA reductase (43) and the LDL receptor (44) provides a potential mechanistic basis for the independent modulation of cholesterol synthesis and receptor-dependent LDL transport by cellular sterols. It should be noted, however, that changes in HMG CoA reductase mRNA levels could account for only a small portion of the increase in hepatic cholesterol synthesis induced by dietary psyllium and cholestyramine. Posttranscriptional regulation of HMG CoA reductase may therefore account for much of the difference in the degree to which these two pathways are regulated in response to depletion of cellular sterols.

The effects of psyllium on liver and plasma cholesterol levels were more impressive in animals fed diets enriched with cholesterol and triglyceride. In these animals, plasma LDL cholesterol concentrations fell from 64 to 33 mg/dl (48%) when psyllium (18 g/1,000 kcal) was substituted for cellulose. Moreover, whereas dietary psyllium had no effect on hepatic LDL receptor expression in animals fed the cholesterol-free, very-low-fat diet, psyllium increased (or restored) receptor expression in animals fed diets enriched with cholesterol and triglyceride. Similarly, the hypocholesterolemic effect of psyllium in humans may be somewhat greater in individuals consuming unrestricted diets (9) than in individuals adhering to American Heart Association Step I diets (10–12). Nevertheless, the greatest reduction in plasma LDL concentrations is achieved by reducing saturated fat and cholesterol in the diet, in addition to increasing soluble fiber (45).

In summary, 7.5% dietary psyllium produced effects on hepatic 7α-hydroxylase, hepatic cholesterol synthesis and LDL metabolism that were similar to those of 1% cholestyramine. Cholestyramine in this dose range clearly increases fecal bile acid excretion (46); however, depletion of the enterohepatic pool of bile acids is largely prevented by derepression of hepatic 7α-hydroxylase so that cholesterol absorption efficiency is not affected (24, 47). Similarly, dietary psyllium has little or no effect on the fecal excretion of neutral steroids or on cholesterol absorption efficiency (8, 20, 24, 47). Together, these studies suggest that dietary psyllium induces a net loss of sterol from the liver that is due largely, if not entirely, to up-regulation of 7α-hydroxylase. Net loss of sterol from the liver, in turn, triggers compensatory responses, some of which may lead to lower circulating LDL levels.

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