Hypolipidemic mechanisms of pectin and psyllium in guinea pigs fed high fat–sucrose diets: alterations on hepatic cholesterol metabolism

Marcela Vergara-Jimenez,[1,*] Karin Conde,[*] Sandra K. Erickson,[†] and Maria Luz Fernandez[*]

Department of Nutritional Sciences,* University of Connecticut, Storrs, CT 06269-4017, and Veterans Affairs Medical Center and Department of Medicine,† University of California, San Francisco, CA 94121-1598

Abstract **Studies were conducted to determine whether pectin (PE) or psyllium (PSY) could reverse the high plasma cholesterol and triacylglycerol (TAG) concentrations induced by high fat (HF) or high sucrose (HS) diets and which are the mechanisms involved. Male guinea pigs were fed either a low fat (LF) or a HF diet with 80% of the carbohydrate energy derived from sucrose. Cellulose was used as control. Plasma LDL cholesterol, TAG, apolipoprotein B, and hepatic cholesteryl ester were lower in guinea pigs fed PE and PSY compared to the control group ($P < 0.03$). In addition, a 45% higher number of hepatic apoB/E receptors was observed by PE and PSY intake. Hepatic ACAT, HMG-CoA reductase, and cholesterol 7α-hydroxylase (C7H) activities were higher in the HF compared to the LF groups ($P < 0.01$). PSY intake with HF resulted in up-regulation of C7H and HMG-CoA reductase activities ($P < 0.05$). Additional studies measuring the effects of PE and PSY on low density lipoprotein (LDL) transport and very low density lipoprotein (VLDL) secretion were conducted in the HF groups. ApoB secretion was reduced by pectin and psyllium ($P < 0.01$) intake while LDL fractional catabolic rates were 100% faster in guinea pigs fed PE or PSY. In these studies the extent of the hypolipidemic response was specific to each fiber type and associated with the amount of sucrose. In addition, PSY altered the activity of hepatic enzymes of cholesterol homeostasis in the HF group. These additional effects of PSY might explain the more dramatic changes in plasma lipid levels associated with PSY consumption.**—Vergara-Jimenez, M., K. Conde, S. K. Erickson, and M. L. Fernandez. **Hypolipidemic mechanisms of pectin and psyllium in guinea pigs fed high fat–sucrose diets: alterations on hepatic cholesterol metabolism.** ***J. Lipid Res.*** **1998.** 39: **1455–1465.**

Supplementary key words pectin • psyllium • dietary fat amount • sucrose • apoB/E receptor • HMG-CoA reductase • cholesterol 7α-hydroxylase • guinea pig

The cholesterol- and triacylglycerol (TAG)-lowering effects of dietary soluble fiber have repeatedly been observed in animal (1) and human (2) studies. It is well known that elevated levels of plasma cholesterol and plasma TAG are associated with the development of coronary heart disease (CHD).

Low fat/high carbohydrate (CHO) diets, especially simple CHO diets, are known to increase plasma TAG concentrations (3, 4) while high fat/low CHO diets have been correlated with higher plasma LDL cholesterol levels (5, 6). Studies in guinea pigs demonstrated that sucrose intake increases VLDL apoB secretion rates and plasma cholesterol concentrations compared to complex CHO intake (7). In contrast, dietary soluble fiber has been shown to lower plasma LDL cholesterol levels in guinea pigs (8, 9). Although there are numerous studies relating the beneficial effects of soluble fiber in lowering plasma cholesterol concentrations, little is known related to its potential use in lowering plasma TAG concentrations induced by high sucrose intake. Thus these studies were undertaken to further explore the interactive effects of dietary soluble fiber and hyperlipidemic nutrients such as fat and sucrose.

Psyllium (PSY), a hydrophilic gel-forming polymer, is currently used in the management of diverticular diseases and functional disorders of the large bowel. PSY apparently exerts its hypocholesterolemic mechanism by enhancing hepatic cholesterol utilization by its biodegradation into bile acids in hamsters (10) and similar observations have been documented in human studies (11). In addition, secondary mechanisms such as reduction of apoB secretion rates or increases in LDL receptor-mediated catabolism have been observed in guinea pigs fed PSY (8).

Pectin (PE), another source of soluble fiber, reduces

Abbreviations: PE, pectin; PSY, psyllium; CON, control; CHO, carbohydrate; HF, high fat; LF, low fat; LDL, low density lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein; apo, apoprotein; RID, radioimmunodiffusion; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; ACAT, acyl-CoA:cholesterol acyltransferase; C7H, cholesterol 7α-hydroxylase; FC, free cholesterol; CE, cholesteryl ester; TAG, triacylglycerol; PL, phospholipids; LCAT, lecithin cholesterol acyltransferase, FCR, fractional catabolic rate.

[1] To whom correspondence should be addressed.

Downloaded from www.jlr.org by guest, on June 14, 2012

cholesterol absorption in guinea pigs compared to guinea pigs fed a cellulose diet (control) (9). This primary action in the small intestine results in plasma VLDL and LDL cholesterol lowering in response to depletion of hepatic cholesterol and induction of apoB/E receptors (12, 13).

Certain primary and secondary mechanisms responsible for the lowering of plasma LDL cholesterol due to specific physiological alterations induced by PE and PSY have been documented in guinea pigs (9, 12). Therefore, in these studies, the hypothesis to be tested was that PE and PSY would reverse the elevated plasma lipid concentrations associated with the intake of diets containing 0.17% cholesterol and 80% of total CHO energy derived from sucrose by distinct mechanisms associated with each specific source of soluble fiber. This level of dietary cholesterol is equivalent to an absorbed amount equal to the daily synthesis rates in guinea pigs (14). In addition, these studies address the interaction of PE and PSY with diets containing low or high fat contributing to 13.3 and 43.2% of the total calories, respectively.

For these studies, guinea pigs were chosen as the animal model due to their similarities to humans in terms of plasma lipoprotein profile, high LDL relative to HDL, distribution of hepatic cholesterol pools, and similar response to dietary factors such as fat, CHO, and dietary soluble fiber (9, 12).

MATERIALS AND METHODS

Materials

dl-Hydroxy-[3-^{14}C]methylglutaryl coenzyme A (1.81 GBq/mmol), dl-[5-^{3}H]mevalonic acid (370 GBq/mmol), cholesteryl-[1,2,6,7-^{3}H]oleate (370 GBq/mmol), Aquasol, Liquiflor (toluene concentrate) and [^{14}C]cholesterol were purchased from DuPont NEN (Boston, MA). Oleoyl-[1-^{14}C]coenzyme A (1.8 GBq/mmol), ^{125}I, and dl-3-hydroxy-3-methylglutaryl coenzyme A were purchased from Amersham (Clearbrook, IL). Cholesteryl oleate, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, NADP, Tyloxapol (Triton WR-1339), triacylglycerol kits, and bovine albumin were from Sigma Chemical (St. Louis, MO). Enzymatic cholesterol assay kits, cholesterol oxidase, cholesterol esterase, and hydroperoxidase were purchased from Boehringer Mannheim (Indianapolis, IN) and halothane was from Halocarbon (Hackensack, NJ). Free cholesterol and phospholipid enzymatic kits were from Waco Pure Chemical (Osaka, Japan). Quick-Seal tubes were from Beckman (Palo Alto, CA). High methoxylated pectin from lime peels and containing 6.7% methoxyl groups and 74% galacturonic acid was obtained from Grinsted Products Inc. (Industrial Airport, KY); and powdered psyllium husk #40-purified 95% and containing less than 3% fat and 1% protein was obtained from Meer Corporation (North Bergen, NJ).

Diets

Diets were prepared and pelleted by Research Diets (New Brunswick, NJ). Six different diets were used during these studies, low fat and high fat diets. The amount of protein, fiber, vitamins, minerals, and cholesterol in these diets was adjusted to the same energy density. Diets differ in the fiber source and the amount of fat and carbohydrate (**Table 1**). Dietary cholesterol was adjusted to 0.42 g/1000 kcal which is equivalent to an amount of absorbed cholesterol equal to the daily synthesis rates in guinea pigs (14). Three different types of fiber were tested; cellulose, a source of insoluble fiber (control diet), and two sources of soluble fiber, pectin (PE) and psyllium (PSY). The low fat diets contained 19.5 g/100 g protein (22.7% of total energy); 55 g/100 g carbohydrates (CHO) equivalent to 64% of total energy, with sucrose contributing to 80% of the CHO energy and 5 g/100 g (13.3 % energy) of a fat mix with a 2:1:1 ratio of saturated:monounsaturated:polyunsaturated fatty acids (23.8 g/100 g lauric, 7.8 g/100g myristic, 9.2 g/100 g palmitic, 8.6 g/100g stearic, 19.9 g/100g oleic, and 26.4 g/100 g linoleic). The fat mix was formulated with 24% olive oil, 49% palm kernel oil, and 27% safflower oil. The high fat diets contained 22.8 g/100 g protein, 19 g/100 g fat, and 35 g/100 g total CHO (34.9% of total energy) with 80% of the CHO energy contributed by sucrose. As PE and

TABLE 1. Composition of experimental diets

Nutrient	Low Fat Diets			Energy	High Fat Diets			Energy
				%				%
Protein	19.5	19.5	19.5	22.7	22.8	22.8	22.8	23.0
Fat mix[a]	5.1	5.1	5.1	13.3	19.0	19.0	19.0	43.2
Starch	11.0	11.0	11.0	12.8	7.0	7.0	7.0	7.0
Sucrose	44.0	44.0	44.0	51.2	28.0	28.0	28.0	27.9
Vitamins[b]	0.9	0.9	0.9	—	1.0	1.0	1.0	1.0
Minerals[b]	7.1	7.1	7.1	—	8.2	8.2	8.2	—
Cellulose	**12.0**	—	4.8	—	**14.0**	—	5.6	—
Pectin	—	**12.0**	—	—	—	**14.0**	—	—
Psyllium	—	—	**7.2**	—	—	—	**8.4**	—
Cholesterol	0.15	0.15	0.15	—	0.17	0.17	0.17	—
Kjoule/g	5.18	5.18	5.18	—	5.97	5.97	5.97	—
Nutrient caloric density (g/1000 kcal)								
Fiber	34.9	34.9	34.9		34.9	34.9	34.9	
Minerals	20.6	20.6	20.6		20.6	20.6	20.6	
Vitamins	2.60	2.60	2.60		2.60	2.60	2.60	
Proteins	57.3	57.3	57.3		57.3	57.3	57.3	
Cholesterol	0.42	0.42	0.42		0.42	0.42	0.42	

[a] Fat mix with polyunsaturated/saturated ratio of 0.5 with 2:1:1 saturated, monounsaturated, and polyunsaturated fatty acids (23.8% lauric, 7.8% myristic, 9.2% palmitic, 8.6% stearic, 19.9% oleic, and 26.4% linoleic).

[b] Vitamins and minerals were formulated to meet NRC specified requirements for guinea pigs. A detailed composition of vitamins and minerals has been reported elsewhere (14)

Downloaded from www.jlr.org by guest, on June 14, 2012

PSY have similar hypocholesterolemic effects when pectin is given as the sole fiber source and PSY at a concentration of 60 g/100 g total soluble fiber (10, 12), the amounts of fiber were adjusted accordingly in the low and high fat diets.

Animals

Male Hartley guinea pigs (Sasco Sprague Dawley, Omaha, NE), weighing 300–400 g (six per group) were randomly assigned to one of six dietary groups for 4 weeks. Preliminary studies have shown that this time (4 weeks) is sufficient to establish a constant plasma cholesterol concentration and a metabolic steady state (9). Animals were housed in a room with a controlled light cycle (light 0700–1900 h) and provided with free access to the semipurified diets and water. Animals were anesthetized with halothane and killed by heart puncture. Blood was utilized for the measurement of plasma lipids and lipoproteins. Hepatic tissue was harvested for the isolation of microsomes and for the measurement of hepatic lipids. For the animals involved in the TAG secretion rate assay, food was withheld for 12 h prior to killing to reduce postprandial plasma TAG concentrations. Guinea pigs used for the in vivo studies were anesthetized with ketamine (0.33 g/kg body weight) for implantation of a catheter, and experiments were conducted 4 to 5 h after surgery. Animals used in kinetic studies were killed by an excess of halothane vapors and those used for TAG secretion rate studies were killed by exsanguination by cardiac puncture to obtain plasma for isolation of nascent VLDL. All animal experiments were conducted in accordance with U.S. Public Health Services and U.S. Department of Agriculture guidelines, and experimental protocols were approved by the Institutional Animal Care and Use Committee.

Plasma and liver lipids

Total plasma and lipoprotein cholesterol concentrations were determined by enzymatic analysis (15). VLDL + IDL and LDL were separated by sequential ultracentrifugation in an L8-M ultracentrifuge (Beckman Instruments, Palo Alto, CA) at 125,000 *g* at 15°C for 19 h in a Ti-50 rotor. Separation was based on the following density fractions: d < 1.019 kg/L for VLDL and IDL, and for LDL d < 1.019–1.090 kg/L. In addition, plasma HDL cholesterol was determined using the precipitation method of Warnick, Bederson, and Albers (16).

Hepatic total and free cholesterol and TAG were determined according to Carr, Andressen, and Rudel (17) after extraction of hepatic lipids with chloroform–methanol 2:1. Hepatic cholesteryl ester concentrations were calculated by subtracting hepatic free from total cholesterol.

VLDL and LDL characterization

VLDL and LDL composition was calculated by determining free and esterified cholesterol (17), protein by a modified Lowry procedure (18), and TAG and phospholipids by use of enzymatic kits. VLDL apoB was selectively precipitated with propanol-2 (19). The number of constituent molecules of VLDL and LDL was calculated on the basis of one apoB per LDL (purity confirmed by SDS-PAGE) with a molecular mass of 412,000 kD (20). The molecular weights were: 885.4, 386.6, 645, and 734 for TAG, free and esterified cholesterol, and phospholipids, respectively.

Determination of apoB concentrations

Polyclonal antibodies against apoB-100 were prepared by injecting guinea pig purified LDL (checked by SDS-PAGE) into a sheep in one dose (300 mg/L) followed by two booster doses (200 mg/L) every 10 days. Antibodies were purified by use of antigen affinity column and apoB antibodies were eluted by modification of pH (21). The specificity of the polyclonal antibodies was tested by use of radioimmunodiffusion (RID) kits. ApoB concentrations in plasma were measured by silver-enhanced radioimmunodiffusion (SERID) in which the antigen was allowed to diffuse radially from wells punched into gel media containing the antibody (22). RID plates were incubated at 37°C for 72 h. Diameters of the immunoprecipitate were read using an RID reader. Linear regression equations were generated for the standard calibrator curve to calculate sample concentrations. The standards were prepared by isolating guinea pig LDL at a more restricted density (d 1.023–1.075 kg/L) and further purification by use of agarose column chromatography. The purity of LDL was checked by SDS-PAGE. Protein was determined by a modification of the Lowry method (18) and standards ranging in concentration from 0 to 650 mg/L were prepared.

LCAT assay

Freshly isolated plasma samples were incubated at 0°C (control) or 37°C (experimental) for 6 h. Plasma free cholesterol concentrations were determined in control and incubated samples by enzymatic methods using a microtiter plate reader (17). This method measures the physiological plasma LCAT activity from the decrease in endogenous free cholesterol in incubated plasma samples. LCAT activity is expressed as the molar cholesterol esterification rate (μmol decrease in unesterified cholesterol/h per L plasma). The conditions for plasma LCAT activity have been standardized for guinea pigs (8).

Isolation of hepatic microsomes

Guinea pigs were killed and livers were removed for the isolation of hepatic microsomes. Isolation of hepatic microsomes was performed by pressing liver tissues through a tissue grinder into 1:3 homogenization buffer (50 μmol/L KH_2PO_4, 0.1 mol/L sucrose, 50 μmol/L NaCl, 30 μmol/L EDTA, and 2 μmol/L dithiothreitol, pH 7.2). This preparation was further homogenized with a Potter-Elvehjem homogenizer. A microsomal fraction was isolated by two 25-min centrifugations at 10,000 *g* (JA-20 rotor, J2-21) followed by ultracentrifugation at 100,000 *g* in a Ti-50 rotor at 4°C. Microsomes were resuspended in the homogenization buffer and centrifuged an additional hour at 100,000 *g*. After centrifugation, microsomal pellets were homogenized and stored at −70°C. Hepatic microsomes were utilized to measure 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), acyl-CoA cholesterol acyltransferase (ACAT), and cholesterol 7α-hydroxylase (C7H) activities.

Hepatic HMG-CoA reductase assay

Microsomal HMG-CoA reductase (EC 1.1.1.34) activity was measured as described by Shapiro, Imblum, and Rodwell (23). Briefly, 200 μg of microsomal protein was incubated with 7.5 nmol (0.33 GBq/nmol) [3-^{14}C]HMG-CoA, 4.5 mol glucose-6-phosphate, 3.6 μmol EDTA, 0.45 μmol NADP, and 0.3 IU glucose-6-phosphate dehydrogenase for 15 min at 37°C to a final volume of 0.05 mL. [^{3}H]mevalonic acid used as an internal recovery standard (0.025 ml per assay) was added to stop the reaction and 1.2 kg/L of unlabeled mevalonate was added to increase recovery. Samples were further incubated at 37°C for 30 min. After incubation, microsomal protein was precipitated by centrifugation for 1 min, and an aliquot of the supernatant was applied to silica gel TLC plates (Alltech, Deerfield, IL). Plates were developed in acetone–benzene 1:1, and the area containing the mevalonate (R_f 0.6–0.9) was scraped and mixed with 5 ml of Aquasol. Radioactivity was measured using a scintillation counter. HMG-CoA reductase activity was expressed as picomoles of [^{14}C]mevalonate produced per min per milligram of microsomal protein. Recoveries of [^{3}H]mevalonate were between 65 and 80%.

Downloaded from www.jlr.org by guest, on June 14, 2012

Hepatic ACAT assay

Hepatic ACAT (EC 2.3.1.26) activity was determined by preincubating microsomal protein (0.8 to 1 mg per assay) with 84 g/L, the amount of albumin equivalent to the molar ratio of the substrate (1:1 albumin:[^{14}C]oleoyl-CoA)(24), and buffer (50 μmol/L KH_2PO_4, 8 mol/L sucrose, 50 μmol/L KCl, 30 μmol/L EDTA, and 50 μmol/L NaF) to a final volume of 0.18 mL. After 5 min at 37°C, 20 μl (500 μmol/L) of oleoyl-[1-^{14}C]coenzyme (0.15 GBq/pmol) was added, and the reaction proceeded for 15 min at the same temperature. The reaction was stopped by the addition of 2.5 mL of chloroform–methanol 2:1. The [^{3}H]cholesteryl oleate recovery standard (0.045 GBq per assay) was added, mixed, and allowed to stand overnight at room temperature. The aqueous phase was removed, and after evaporation of the organic phase to dryness, samples were resuspended in 150 μL of chloroform containing 30 μg of unlabeled cholesteryl oleate. Samples were applied to glass silica gel TLC plates (Alltech) and developed in hexane–diethyl ether 9:1. Cholesteryl oleate was visualized with iodine vapors and scraped from the TLC plates, 5 ml of liquiflour was added, and radioactivity was counted in a scintillation counter. Recoveries of the [^{3}H]cholesteryl oleate ranged from 75 to 90%.

Hepatic cholesterol 7α-hydroxylase assay

Cholesterol 7α-hydroxylase (EC 1.14.13.7) activity was assayed by the method of Shefer, Hauser and Mosbach (25) as modified by Jelinek et al. (26) using [^{14}C]cholesterol as substrate, except that cholesterol was delivered as cholesterol:phosphatidylcholine liposomes (1:8 by weight) prepared by sonication and a NADPH-regenerating system (glucose-6-phosphate dehydrogenase, NADP, and glucose-6-phosphate) was included in the assay. After addition of glucose-6-phosphate dehydrogenase (0.3 IU), samples were incubated for an additional 30 min. The reaction was stopped by addition of 5 mL of chloroform–methanol 2:1 and 1 ml of acidified water (5% sulfuric acid). Tubes were mixed, the top layer was discharged, and samples were dried under nitrogen. Samples and 7α- and 7β-hydroxycholesterol standards each were dissolved in 100 μl of chloroform, applied to silica gel TLC plates, and developed with ethyl acetate–toluene 3:2. The plates were exposed to iodine vapors to mark the 7α and 7β-hydroxycholesterol standards and then placed on XAR-5 film with intensifying screen overnight. Using the film as a guide, the location of the [^{14}C]7α-hydroxycholesterol spots was determined and the gel was scraped from the plate. Five ml of liquiflour was added to the gel, and the mixture was counted in a scintillation counter.

LDL binding assay

Pooled samples of guinea pig LDL were radioiodinated with ^{125}I by the iodine monochloride method (27) to give a specific activity between 100–300 cpm/ng. Hepatic microsomes were incubated with the radiolabel LDL over a concentration range of 10 to 80 μg/mL in the presence or absence of 1 mg/mL of unlabeled human LDL, an effective competitor at 37°C to determine total and non-specific binding. Receptor-mediated binding was determined by difference. After incubation for 2 h at 37°C, microsomes were pelleted by ultracentrifugation in a Ti 42.2 rotor at 100,000 *g* for 1 h, followed by a washing of 30 min with 100 μL of 3 g/100 g albumin solution. Tubes were sliced at the bottom and counted in a scintillation counter. K_d and B_{max} were determined from Woolf plots (28).

Triacylglycerol secretion rate

The rates of VLDL TAG and apoB secretions were determined by blocking VLDL catabolism with Triton WR-1339, a detergent that coats VLDL particles, thus impeding the action of lipoprotein lipase (LPL) in vivo. This method has been well characterized in guinea pigs (29, 30). Food was withheld from animals 12 h prior to surgery and a catheter was inserted into the carotid artery for injection of Triton and continuous blood sampling. Guinea pigs were fasted during the 8 h of the experiment to ensure that the measured plasma TAG levels reflected VLDL secretion and not influx of dietary TAG as chylomicrons. A 20% Triton solution (100 mg/kg of body weight) was injected and blood samples (500 μl) were taken at 0, 5, 10, 15, 20, 50, 75, 120, 180, 300, and 480 min. Plasma was separated from red blood cells and TAG concentrations were measured for each time point. TAG accumulation in plasma increased linearly with time. TAG secretion rate was calculated by regression as mmol TAG secreted/(kg body weight·h). Calculations were based on the assumption that plasma equals 4.0% of body weight in guinea pigs (31). ApoB secretion rates were calculated by multiplying VLDL TAG secretion rate × apoB concentrations (%) divided by VLDL TAG (%) (7).

Isolation and characterization of VLDL

At the end of the TAG secretion rate experiments, guinea pigs were killed and nascent VLDL was isolated (d 1.006 kg/L); composition was determined by measuring protein, TAG, phospholipids, and cholesterol as previously reported (7). VLDL apoB was selectively precipitated with propanol-2 (32); protein was determined in the supernatant and VLDL apoB was calculated by difference. The number of component molecules of nascent VLDL was calculated as was mentioned above for the VLDL and LDL characterization.

In vivo LDL kinetics

Guinea pig plasma LDL (1.019–1.09 kg/L) was isolated by ultracentrifugation from guinea pigs fed the three high fat diets. This plasma was for injection into recipient animals from the same dietary group to mimic the in vivo situation and not to introduce another variable such as changes in affinity of LDL receptors of the recipient animals to LDL particles modified by different dietary treatments. Centrifugation was performed in a Ti 50 rotor for 24 h at 100,000 *g* at 15°C. LDL was dialyzed against 0.9 g NaCl + 0.01 g EDTA/100 g water for 24 h and iodinated according to Goldstein, Basu, and Brown (27) using ^{125}I. ^{125}I-labeled LDL was used within 2 days of iodination to minimize damage due to radiation oxidation. In LDL 95-97% of the radioactivity was associated with apoB while 3–5% of the radioactivity was associated with free iodine.

Animals were injected with the isotope through an indwelling catheter inserted via carotid artery, and plasma samples were taken at 0, 1, 3, 10, 40, and 60 min and at 3, 5, 10, 22, and 28 h (9). The plasma disappearance of radiolabeled LDL was followed by counting plasma samples directly in the gamma counter, and LDL fractional catabolic rates (FCR) were determined by use of a two-pool model as described by Matthews (33).

Statistical analysis

Two-way analysis of variance (ANOVA) (GBSTAT, Silver Spring MD) was used to test significant fat and fiber effects on plasma lipids, hepatic cholesterol, and TAG, weight gain, plasma apoB, TAG secretion rate, composition of nascent VLDL and LDL turnover studies, apoB/E receptor B_{max} and K_d and activities of hepatic HMG-CoA reductase, ACAT, and cholesterol 7α-hydroxylase. The Tukey's test was used as post hoc test to evaluate differences among means when an interactive effect was present. Because animals fed the PSY diet gained less weight as a group than control animals or those fed PE, an analysis of covariance was performed using plasma cholesterol as the dependent variable and weight as the covariate to determine whether or not the observed differences were associated with changes in weight gain. Regression analysis was used to identify significant correla-

Downloaded from www.jlr.org by guest, on June 14, 2012

tions. One-tail Student's *t*-test was used to calculate differences between control and soluble fiber in the FCR and apoB secretion rates. Statistical analysis of the kinetic data was fitted using a two-pool model (JANA, SCI Software, Lexington, KY). Data are presented as the mean ± SD for the number of animals tested. Differences were considered significant at $P < 0.05$.

RESULTS

Effects of PE and PSY with low and high fat diets on plasma lipid and lipoproteins

Guinea pigs fed the PSY diet had lower weight gain than those in the other two dietary groups. However, by performing an analysis of covariance, no correlation between plasma cholesterol and weight gain was found for any of the dietary groups ($P = 0.445$) and the significant differences in plasma cholesterol persisted after using weight gain as a covariate ($P = 0.002$)(**Table 2**). Plasma cholesterol concentrations were affected both by dietary fiber and the amount of fat intake ($P < 0.001$). Guinea pigs fed PE and PSY had 35% lower plasma cholesterol compared to the control in the low fat group and 30–60% lower than those in the high fat group (Table 2). Guinea pigs fed the PSY diet had the lowest plasma cholesterol concentrations (interaction effect, $P < 0.01$). Plasma TAG concentrations were 40% lower as a result of intake of PE and PSY in the high fat group only ($P < 0.05$) while no differences in plasma TAG levels were observed for the three dietary fiber groups (cellulose, PE, and PSY) in the low fat group (Table 2). Guinea pigs fed the high fat diets had higher plasma cholesterol and TAG concentrations than those from the low fat group ($P < 0.001$).

PE and PSY intake resulted in lower plasma apoB concentrations ($P < 0.01$) which were more pronounced in guinea pigs fed the high fat diet ($P < 0.05$) (Table 2).

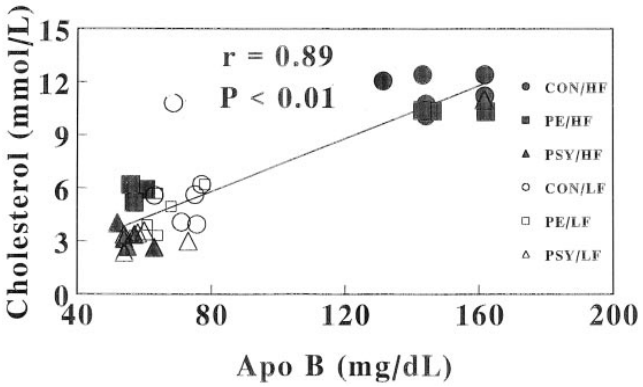

Fig. 1. Correlation between plasma cholesterol concentrations and plasma apoB ($r = 0.89$, $P < 0.01$) of guinea pigs fed cellulose / high fat (con / hf), pectin / high fat (pe / hf), psyllium / high fat (psy / hf), cellulose / low fat (con / lf), pectin / low fat (pe / lf) or psyllium / low fat (psy / lf). Each point represents an individual guinea pig.

Guinea pigs fed the high fat diets had more elevated plasma apoB concentrations than those from the low fat group ($P < 0.001$). There was a positive correlation between total plasma cholesterol and apoB ($r = 0.89$, $P < 0.01$) for all dietary groups (**Fig. 1**), which suggests a decrease in the number of apoB-containing particles in guinea pigs fed low fat diets and this decrease apparently contributes to the reduction in total plasma cholesterol in the low fat compared to the high fat groups.

VLDL cholesterol concentrations were not affected by dietary treatments. In contrast, plasma LDL cholesterol concentrations were lower in animals fed PE and PSY ($P < 0.001$) compared to control (**Table 3**) and PSY had a greater LDL cholesterol lowering in the high fat group (interaction effect, $P < 0.05$). Animals in the high fat group had higher plasma LDL concentrations than ani-

TABLE 2. Weight gain, plasma lipids, and apolipoprotein B of guinea pigs fed low fat (5.5%) or high fat (19%) diets with 64 or 35% of total energy derived from carbohydrates (CHO), respectively, and 80% of CHO energy derived from sucrose

Diets	Weight Gain	Cholesterol	Triacylglycerol	ApoB
	g/4 wk	*mmol/L*	*mmol/L*	*mg/dL*
Low Fat				
Control	210 ± 21	4.03 ± 1.68	0.58 ± 0.12	72 ± 5
Pectin	204 ± 27	3.12 ± 0.87	0.68 ± 0.09	65 ± 7
Psyllium	178 ± 27	2.09 ± 0.34	0.68 ± 0.24	60 ± 8
High Fat				
Control	212 ± 39	7.68 ± 0.62	1.30 ± 0.24	148 ± 12
Pectin	199 ± 14	5.40 ± 1.71	0.90 ± 0.16	104 ± 51
Psyllium	127 ± 33	2.99 ± 2.17	0.79 ± 0.17	74 ± 43
Two-way ANOVA				
Fat amount[a]	NS	$P < 0.001$	$P < 0.001$	$P < 0.001$
Fiber type[b]	$P < 0.001$	$P < 0.001$	$P < 0.05$	$P < 0.01$
Interaction	NS	$P < 0.05$	$P < 0.001$	$P < 0.05$

Cellulose (control), pectin or psyllium were used as sources of dietary fiber. Data are presented as mean ± SD for 6 guinea pigs.

[a] High fat > low fat for plasma cholesterol, triacylglycerol and apoB.

[b] Psyllium < pectin = control for weight gain. Psyllium = pectin < control for plasma cholesterol, triacylglycerol, and apoB.

TABLE 3. Plasma VLDL, LDL, and HDL cholesterol concentrations of guinea pigs fed low fat (5.5%) or high fat (19%) diets with 64 or 35% of total energy derived from carbohydrates (CHO), respectively, and 80% of CHO energy derived from sucrose

	Plasma Lipoproteins		
Diets	VLDL	LDL	HDL
	mmol/L		
Low fat			
Control	0.18 ± 0.28	3.65 ± 1.63	0.18 ± 0.05
Pectin	0.13 ± 0.21	2.74 ± 0.80	0.18 ± 0.03
Psyllium	0.10 ± 0.02	1.84 ± 0.34	0.21 ± 0.05
High fat			
Control	0.31 ± 0.23	6.78 ± 0.41	0.35 ± 0.16
Pectin	0.13 ± 0.05	4.99 ± 1.66	0.31 ± 0.26
Psyllium	0.21 ± 0.31	2.59 ± 1.86	0.21 ± 0.05
Two-way ANOVA			
Fat amount[a]	NS	$P < 0.001$	$P < 0.05$
Fiber type[b]	NS	$P < 0.001$	NS
Interaction	NS	$P < 0.05$	NS

Cellulose (control), pectin or psyllium were used as sources of dietary fiber. Data are presented as mean ± SD for 6 guinea pigs.

[a] High fat > low fat for HDL.

[b] Psyllium = pectin < control for LDL.

Downloaded from www.jlr.org by guest, on June 14, 2012

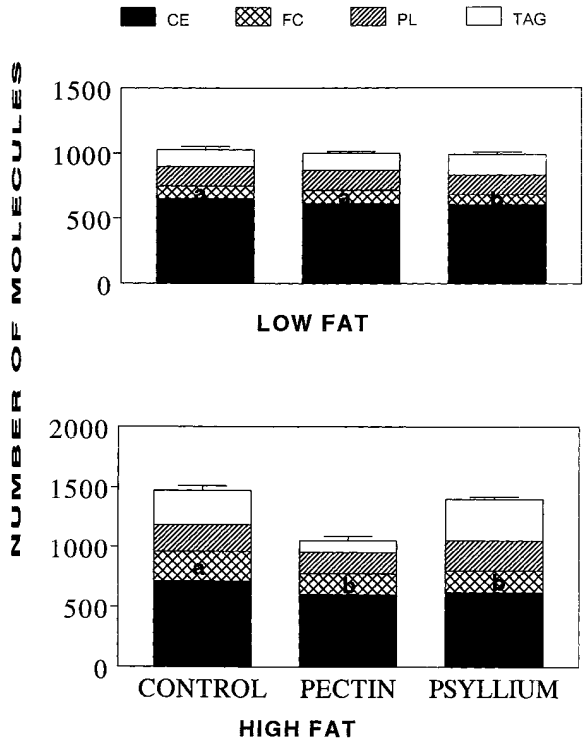

Fig. 2. Number of cholesteryl ester (CE), free cholesterol (FC), phospholipids (PL), and triacylglycerol (TAG) molecules of plasma low density lipoprotein (LDL) particles from guinea pigs fed cellulose/high fat (control), pectin/high fat (pectin), psyllium/high fat (psyllium) (lower panel); and guinea pigs fed cellulose/low fat (control), pectin/low fat (pectin), psyllium/low fat (psyllium) (upper panel). Different superscripts a, b, indicate significantly different. Pectin and psyllium are different from control in the high fat groups and psyllium in the low fat groups is different from control and pectin for the number of free cholesterol molecules in the LDL particle.

mals in the low fat group ($P < 0.001$) (Table 3). Plasma HDL cholesterol concentrations were higher in the high fat groups ($P < 0.05$). PE and PSY had no effect on this parameter.

LDL composition was modified by fat amount and soluble fiber. LDL isolated from animals fed low fat diets and PSY had fewer free cholesterol molecules (**Fig. 2**, upper panel) while PE and PSY intake resulted in a lower number of free cholesterol molecules in LDL isolated from the high fat groups (Fig. 2, lower panel).

Plasma LCAT activity was affected only by dietary fat amount. Guinea pigs fed high fat diets had higher LCAT activity than those fed low fat. As LCAT is involved in the esterification rates of HDL cholesterol, this may be correlated with more elevated plasma HDL cholesterol concentrations in guinea pigs fed high fat. There were no PE, PSY, or interaction effects on LCAT activity (**Table 4**).

TABLE 4. Plasma LCAT activity of guinea pigs fed low fat (5.5%) or high fat (19%) diets with 64 or 35% of total energy derived from carbohydrates (CHO), respectively, and 80% of CHO energy derived from sucrose

Diets	LCAT Activity
	mol/L-h
Low fat	
Control	3.8 ± 2.5
Pectin	4.0 ± 3.2
Psyllium	1.8 ± 1.6
High fat	
Control	12.7 ± 7.3
Pectin	10.4 ± 7.5
Psyllium	7.2 ± 1.0
Two-way ANOVA	
Fat amount[a]	$P < 0.01$
Fiber type	NS
Interaction	NS

Cellulose (control), pectin or psyllium were used as sources of dietary fiber. Data are presented as mean ± SD for 6 guinea pigs.
[a] High fat > low fat for LCAT activity.

Effects of PE and PSY with low and high fat diets on hepatic lipids

Hepatic total cholesterol concentrations were altered by the amount of fat and the type of fiber fed to the guinea pigs (**Table 5**). While hepatic free cholesterol was lower in guinea pigs fed low fat, hepatic cholesteryl ester concentrations were reduced by PE and PSY intake ($P < 0.001$). Hepatic cholesteryl ester concentrations were 62% lower in guinea pigs fed PE and PSY ($P < 0.001$) in the low fat group, and 71% lower in those fed PSY in the high fat group (Table 5). Hepatic TAG were 48% lower in guinea pigs fed PE and PSY in the higher fat group compared to the control group (interaction effect $P < 0.001$) while no changes in hepatic TAG were observed for low fat intake.

Effects of PE and PSY with low and high fat diets on hepatic apoB/E receptors

Hepatic microsomes were used for the measurement of apoB/E receptor number. Results are presented for dietary soluble fiber groups compared to control. Soluble fiber intake resulted in higher number of hepatic apoB/E receptors in both the low and high fat groups compared to control animals by 45% and 41% respectively (**Table 6**). These results suggest that an increase in the number of receptors contributes to a decrease in plasma LDL cholesterol due to hepatic uptake efficiency.

Effects of PE and PSY with low and high fat diets on hepatic enzyme activities

The activity of hepatic enzymes related to cholesterol metabolism was affected by the different dietary treatments. ACAT activity was not altered by PE or PSY; however, there was a clear effect due to the amount of dietary fat on this enzyme activity (**Table 7**).

HMG-CoA reductase activity was higher in animals fed the high fat diet ($P < 0.001$). PSY up-regulated HMG-CoA reductase activity by 55% only in combination with high fat intake (interaction effect $P < 0.05$)(Table 7). C7H activity was higher in guinea pigs fed high fat diets compared to those in the low fat group. In the high fat group, PSY increased the activity of C7H by 24% compared to guinea pigs fed either cellulose or pectin (interaction ef-

Downloaded from www.jlr.org by guest, on June 14, 2012

TABLE 5. Hepatic lipids of guinea pigs fed low fat (5.5%) or high fat (19%) diets with 64 or 35% of total energy derived from carbohydrates (CHO), respectively, and 80% of CHO energy derived from sucrose

Diets	Hepatic Lipids			
	Total Cholesterol	Free Cholesterol	Esterified Cholesterol	Triacylglycerol
	μmol/g			
Low Fat				
Control	9.57 ± 2.33	5.43 ± 1.03	4.14 ± 1.81	1.24 ± 0.11
Pectin	8.02 ± 3.36	5.69 ± 1.81	2.33 ± 1.81	3.28 ± 1.92
Psyllium	5.43 ± 0.78	4.40 ± 0.52	0.78 ± 0.52	2.48 ± 1.36
High fat				
Control	11.38 ± 3.10	7.24 ± 1.55	4.40 ± 1.81	4.18 ± 1.81
Pectin	9.31 ± 2.33	6.21 ± 1.81	3.36 ± 1.03	2.48 ± 1.36
Psyllium	7.50 ± 2.33	6.21 ± 2.59	1.29 ± 0.52	1.81 ± 1.11
Two-way ANOVA				
Fat amount[a]	$P < 0.05$	$P < 0.05$	NS	NS
Fiber type[b]	$P < 0.001$	NS	$P < 0.001$	NS
Interaction	NS	NS	NS	$P < 0.001$

Cellulose (control), pectin or psyllium were used as sources of dietary fiber. Data are presented as mean ± SD for 6 guinea pigs.
[a] High fat > low fat for total and free cholesterol.
[b] Psyllium < Pectin = control for total and esterified cholesterol.

fect $P < 0.05$). These results suggest that the effect of PSY on these two regulatory enzymes of cholesterol homeostasis, HMG-CoA reductase and C7H, may be associated with the hypolipidemic action of PSY.

As no changes in plasma TAG concentrations in guinea pigs fed low fat/high CHO diets were demonstrated, a second set of experiments using 12 guinea pigs per dietary group was conducted where only the high fat/low CHO groups were considered to evaluate other parameters involved in lipid metabolism such as TAG and apoB secretion rates and LDL turnover.

High fat diets

Plasma lipids. In agreement with our previous results, plasma cholesterol concentrations were lower in those guinea pigs fed PSY and PE compared to the control group ($P < 0.01$) (**Table 8**) with PSY having a stronger hypocholesterolemic effect than PE. PE and PSY also decreased plasma TAG ($P < 0.002$) compared to animals fed cellulose (Table 8) and no differences between PE and PSY were found for plasma lipids.

VLDL-TAG and apo-B secretion rates. Hepatic TAG secretion rates were measured by evaluating plasma TAG accumulation after intravenous injection of Triton WR-1339 as previously described. Plasma TAG concentrations increased linearly after 8 h for the three dietary groups, which indicates that there was an interruption of the delipidation cascade in plasma (data not shown). TAG and apoB secretion rates were not affected by PE and PSY intakes (**Table**

TABLE 6. LDL binding to hepatic microsomes of guinea pigs fed low fat (5.5%) or high fat (19%) diets with 64 or 35% of total energy derived from carbohydrates (CHO), respectively, and 80% of CHO energy derived from sucrose

Diets	LDL Binding Parameters	
	B_{max}	K_d
	μg/mg microsomal protein	*mg/L*
Low fat		
Control	1.07 ± 0.15[a]	20 ± 3
Soluble Fiber	1.96 ± 0.73[b]	30 ± 2
High fat		
Control	1.22 ± 0.40[a]	35 ± 3
Soluble Fiber	2.07 ± 0.66[b]	39 ± 2

Cellulose (control), pectin or psyllium were used as sources of dietary fiber. Samples from soluble fiber (PE and PSY) were pooled and analyzed with Student's *t* test. Values represent mean ± SD for guinea pigs fed control (n = 4) and soluble fiber (n = 6). Values in the same column with different superscripts are significantly different ($P < 0.05$) as determined by Student's *t*-test.

TABLE 7. Hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, acyl-CoA:cholesterol acyltransferase (ACAT), and cholesterol 7α-hydroxylase (C7H) activities of guinea pigs fed low fat (5.5%) or high fat (19%) diets with 64 or 35% of total energy derived from carbohydrates (CHO), respectively, and 80% of CHO energy derived from sucrose

Diets	Hepatic Enzyme Activity		
	ACAT	HMG-CoA Reductase	C7H
	pmol/min·mg protein		
Low fat			
Control	10 ± 5	1.6 ± 0.5	1.8 ± 0.3
Pectin	9 ± 5	1.5 ± 0.7	1.8 ± 0.3
Psyllium	7 ± 1	1.4 ± 0.3	1.7 ± 0.2
High fat			
Control	23 ± 5	1.1 ± 0.3	2.5 ± 0.6
Pectin	28 ± 14	1.7 ± 0.3	2.7 ± 0.4
Psyllium	23 ± 8	3.3 ± 1.9	3.4 ± 0.7
Two-way ANOVA			
Fat amount[a]	$P < 0.001$	$P < 0.01$	$P < 0.001$
Fiber type	NS	NS	NS
Interaction	NS	$P < 0.05$	$P < 0.05$

Cellulose (control), pectin or psyllium were used as sources of dietary fiber. Data are presented as mean ± SD for 6 guinea pigs.
[a] High fat > low fat for ACAT, HMG-CoA reductase, and C7H activities.

Downloaded from www.jlr.org by guest, on June 14, 2012

TABLE 8. Plasma lipids of guinea pigs fed high fat (19%) diets with 35% of total energy derived from carbohydrates (CHO) and 80% of CHO energy derived from sucrose

Diets	Cholesterol	Triacylglycerol
	mmol/L	
Control	6.86 ± 1.74[a]	1.30 ± 0.24[a]
Pectin	5.21 ± 1.12[a]	0.90 ± 0.16[b]
Psyllium	2.65 ± 0.80[b]	0.80 ± 0.17[b]

Cellulose (control), pectin or psyllium were used as sources of dietary fiber. Data are presented as mean ± SD for 12 guinea pigs. Values in a column with different superscripts are significantly different, $P < 0.05$.

9). However, the rates of apoB secretion were significantly reduced in the soluble fiber groups compared to the cellulose group ($P < 0.05$) indicating that nascent VLDL composition was altered by PE and PSY (**Fig. 3**).

PE significantly increased the number of TAG molecules in nascent VLDL ($P < 0.03$) compared to the control group (**Fig. 4**). In addition, PSY intake decreased the number of cholesteryl ester molecules ($P < 0.03$) and increased the number of phospholipids in nascent VLDL ($P < 0.01$) compared to the PE and control groups (Fig. 4). These results indicate that PE and PSY modify nascent VLDL particles in a different manner but both treatments result in larger VLDL.

LDL turnover. LDL apoB pool size was lower in PSY and PE fed animals compared to control group (**Table 10**) while apoB FCR or flux was not affected by PE and PSY when analyzed individually (Table 10). However, when we compared soluble fiber versus control groups (**Fig. 5**) there was a reduction in LDL FCR ($P < 0.03$), indicating that soluble fiber increases LDL catabolism possibly due to the increases in hepatic apoB/E receptor number observed within this study (Table 6).

DISCUSSION

We have shown in this study that the extent to which PE or PSY influence plasma lipid levels is significantly related to the presence of other nutrients. For example, it is clear that the hypolipidemic effects of PE are somewhat diminished in the presence of diets containing high sucrose levels as in the case of the low fat diets. The interpretation of these results is based on how PE or PSY influence the absorption rate of nutrients in the small intestine and how

TABLE 9. Triacylglycerol secretion rate of guinea pigs fed high fat (19%) diets with 35% of total energy derived from carbohydrates (CHO) and 80% of CHO energy derived from sucrose

Diets	ApoB Secretion Rate	Triacylglycerol Secretion Rate
	mg/kg·h	
Control	4.30 ± 4.40	57.29 ± 23.56
Pectin	1.53 ± 0.73	60.30 ± 33.01
Psyllium	2.80 ± 1.28	68.86 ± 24.81

Cellulose (control), pectin or psyllium were used as sources of dietary fiber. Data are presented as mean ± SD for 6 guinea pigs per dietary treatment.

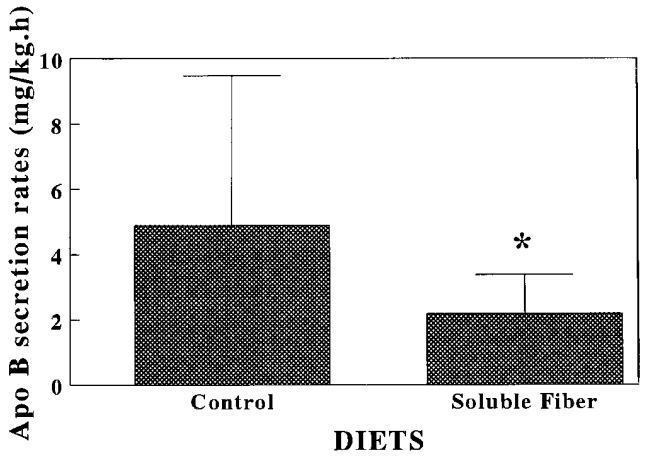

Fig. 3. ApoB secretion rates between guinea pigs fed high fat/pectin, psyllium (soluble fiber) vs. high fat/cellulose (control). Guinea pigs fed soluble fiber significantly reduced ($P < 0.05$) apoB secretion rates compared to guinea pigs fed cellulose. Asterisk (*) indicates significant differences among groups.

these nutrients will affect metabolic routes in the liver and consequently alter secretion and catabolism of VLDL and LDL, the major carriers of TAG and cholesterol, respectively. What is also clear from these studies is that the amount of dietary fat independent of the type of dietary fiber significantly influences hepatic regulatory enzymes of cholesterol homeostasis thereby affecting plasma lipid and lipoprotein concentrations.

PE and PSY intake and plasma lipids and lipoproteins

PE and PSY intake resulted in reductions of plasma cholesterol and TAG concentrations, two major risk factors as-

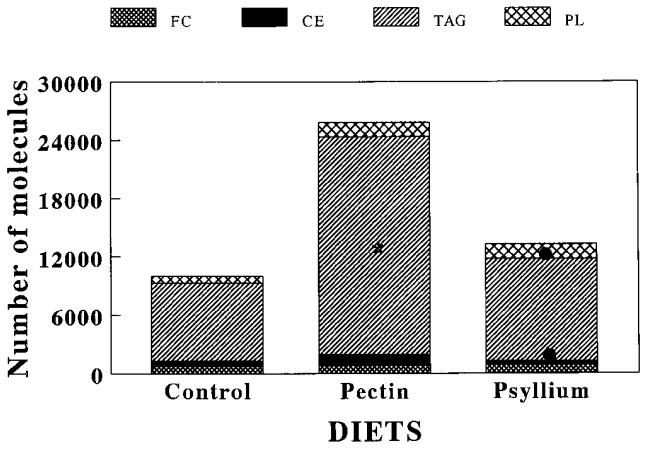

Fig. 4. Number of cholesteryl ester (CE), free cholesterol (FC), phospholipids (PL), and triacylglycerol (TAG) molecules of nascent very low density lipoprotein (VLDL) from guinea pigs fed high fat/cellulose (control), high fat/pectin (pectin), and high fat/psyllium (psyllium). Pectin significantly increased the number of TAG molecules compared to the control and psyllium groups ($P < 0.03$). Psyllium reduced the number of CE molecules ($P < 0.03$) and increased the number of PL molecules ($P < 0.01$) compared to pectin and control groups. Asterisk (*) and closed circles (●) indicate statistical differences among groups.

Downloaded from www.jlr.org by guest, on June 14, 2012

TABLE 10. LDL apoB kinetic parameters of guinea pigs fed high fat (19%) diets with 35% of total energy derived from carbohydrates (CHO) and 80% of CHO energy derived from sucrose

Diets	LDL Kinetics		
	ApoB Pool	ApoB FCR	ApoB Flux
	mg/kg	*pools/h*	*mg/kg-h*
Control	30.00 ± 7.8[a]	0.09 ± 0.02	2.80 ± 1.1
Pectin	20.40 ± 4.9[b]	0.15 ± 0.05	3.10 ± 1.0
Psyllium	14.10 ± 3.2[b]	0.15 ± 0.08	2.00 ± 0.8

Cellulose (control), pectin or psyllium were used as sources of dietary fiber. Data are presented as mean ± SD for 6 guinea pigs. Values in a column with different superscripts are significantly different, $P < 0.05$.

sociated with coronary heart disease. However, the effects of these two soluble fibers were more pronounced with high fat diets which suggests that the higher amount of sucrose in the low fat diets had a negative influence on the hypolipidemic action of PE and PSY.

Similar to the findings by Abbot et al. (34) where lower plasma LDL cholesterol concentrations were observed in subjects consuming low fat compared to high fat diets, intake of low fat resulted in lower plasma LDL cholesterol concentrations in this study. Diets containing PE and PSY improved plasma LDL concentrations even further in agreement with clinical reports where intake of soluble fiber with low fat diets resulted in a more desirable lipoprotein profile (35).

PE and PSY also had an effect on lipoprotein composition and number of molecules. The more pronounced effects of these fibers was observed in combination with high fat diets. Compositional changes in LDL may have important metabolic meaning as reduction in free and esterified cholesterol in LDL have been associated with faster LDL turnover in plasma (36).

LCAT activity was not altered by fiber; however, intake of high fat promoted the conversion of free cholesterol to esterified cholesterol which may be related to the higher plasma HDL cholesterol concentrations observed in guinea pigs fed high fat diets. These results are in agreement with observations by West, Sullivan, and Katan (37) where children from countries consuming high fat diets have higher plasma HDL cholesterol concentrations than those consuming low fat.

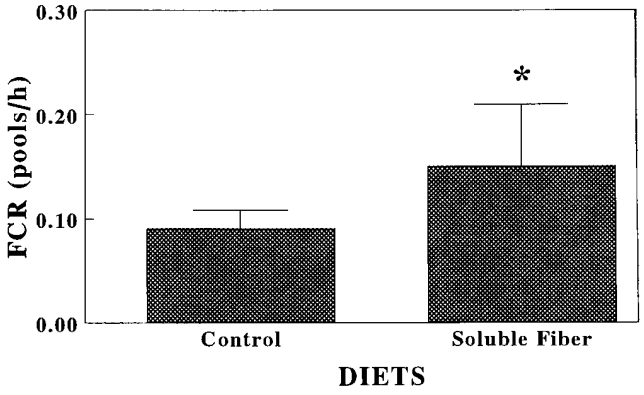

Fig. 5. Low density lipoprotein (LDL) fractional catabolic rate (FCR) between guinea pigs fed high fat/pectin, psyllium (soluble fiber) vs. high fat/cellulose (control). Guinea pigs fed soluble fiber significantly increased their LDL FCR compared to guinea pigs fed cellulose ($P < 0.03$). Asterisk (*) indicates significant differencees among groups.

PE and PSY intake and hepatic cholesterol homeostasis

Hepatic cholesterol concentrations were altered by the amount of fat and the type of fiber fed to guinea pigs. Hepatic cholesteryl ester was lower in guinea pigs fed PE and PSY although the latter had a more pronounced effect. Although we did not find any changes in VLDL cholesterol concentrations, other studies suggest that a decrease in hepatic cholesteryl ester reduces the formation of VLDL particles (38). These alterations of hepatic cholesterol pools have significant implications for the activity of regulatory enzymes of cholesterol metabolism and number of hepatic apoB/E receptors as is discussed below.

Studies in guinea pigs have shown that hepatic apoB/E receptor number is modified by the saturation of dietary fatty acids and that these modifications are highly correlated with plasma LDL catabolism in vivo (39). In these studies, an up-regulation of LDL receptor by soluble fiber was observed independent of the amount of dietary fat, which suggests that a major mechanism by which dietary fiber lowers plasma cholesterol is by augmenting the number of LDL receptors as a response to depletion of hepatic cholesterol pool.

Another important modification induced by PE and PSY in the liver was the reduction in hepatic TAG in guinea pigs fed high fat diets. Studies by Ebihara and Schneeman (40) in the rat demonstrated that the absorption of TAG from the small intestine was delayed due to the feeding of soluble fiber. It is possible that similar decreases in fat absorption took place when PE and PSY were given with high fat diets. This reduction in hepatic TAG could be related to the lower plasma TAG concentrations observed in guinea pigs fed PE and PSY as hepatic TAG pools are related to VLDL secretion rates (37). Another possibility may be associated with the delayed absorption of sucrose components (fructose and glucose) in the intestinal lumen, as suggested in studies conducted by Vuorinen-Markkola, Sinisalo, and Kovisto (41) where hyperlipidemic insulin-dependent diabetic patients were fed guar gum, and a depletion in their fasting blood glucose concentrations was observed.

The amount of fat significantly affected the regulatory enzymes of cholesterol homeostasis in liver. Increases in ACAT activity have been previously reported with high fat intake in guinea pigs (42). In addition, significant correlations between hepatic free cholesterol and ACAT activity have been observed, indicating a relationship between substrate availability and ACAT activity (43). In the present study, animals fed the high fat/PSY diet had higher HMG-CoA reductase activity in agreement with PSY enhancing cholesterol synthesis in hamsters (10).

Cholesterol 7α-hydroxylase (C7H) activity was higher in guinea pigs fed high fat diets and only PSY up-regulated this enzyme activity when given with the high fat diet.

Downloaded from www.jlr.org by guest, on June 14, 2012

These changes are in parallel with the increases in HMG-CoA reductase activity induced by PSY, consistent with the reduction in hepatic free cholesterol concentrations in guinea pigs fed PSY diets. This lower hepatic cholesterol may result from increased production of bile acids due to higher activity of C7H. No alteration of these enzyme activities by PE intake was observed. The discrepancies with our published data (12) and the present findings may be related to the differences in diets as the diets used for these studies contain a high percent of sucrose, and also to the interaction of specific nutrients with PE in the intestinal lumen.

In addition, PE and PSY did not alter enzyme activities in animals fed the low fat diets suggesting that the high levels of sucrose (53% of total energy) in these diets might have obscured the effects of these soluble fibers. In the specific case of guinea pigs fed low fat diets, the addition of soluble fiber did not decrease the rates of esterification of hepatic cholesterol as there already existed a substantial decrease in ACAT activity induced by the lower concentration of fat in the diets, similar to our previous report (42).

PE and PSY intake in secretion and catabolism of apoB-containing lipoproteins

Even though, in these studies, TAG secretion rates were not affected by PE and PSY intake, the rates of apoB secretion were significantly reduced in the soluble fiber groups compared to the control group. Such results indicated that nascent VLDL composition was altered by PE and PSY. Dissociation between VLDL TAG and VLDL apoB production has been observed in subjects consuming high CHO diets (44). These data indicate that the number of particles secreted by the liver can be affected by dietary interventions as VLDL has only one apoB molecule per particle (45). PE increased the number of TAG molecules in nascent VLDL compared to the PSY and control groups. These results suggest that guinea pigs fed PE secrete fewer VLDL particles larger in size and containing more TAG. These modifications in the VLDL particle size may have an effect on the intravascular processing of lipoproteins. It is speculated that larger TAG-enriched particles are a better substrate for lipoprotein lipase which would explain the lower plasma LDL and TAG concentrations observed during PE treatment (46). In addition, PSY intake decreased the number of cholesteryl ester molecules, and increased the number of phospholipids in nascent VLDL compared to the PE and control groups. VLDL particles containing a higher proportion of cholesteryl ester are more easily converted to intermediate density lipoprotein and then to LDL through the delipidation cascade (46). Thus the VLDL derived from the PSY diet might not readily be converted to LDL but rather removed from the circulation through the apoB/E receptor that was up-regulated by PSY intake.

We did not observe an effect on LDL flux caused by fiber intake which suggests that the major effects of PE and PSY when given in combination with high sucrose are more related to increases in LDL catabolism and possibly removal of VLDL from plasma and interruption of the delipidation cascade. The observed increases in LDL apoB FCR induced by PE and PSY are in agreement with other reports in the literature where dietary soluble fiber in the rat (47) and PSY in the hamster (48) have been shown to enhance LDL uptake.

From these studies we can conclude that the interaction of specific nutrients including sucrose, amount of fat, and dietary soluble fiber significantly alter plasma lipid and lipoprotein profile based on alterations occurring in the liver and in the plasma compartment. It may be that the amount of fat and simple CHO intake determined the primary mechanism of soluble fiber action in the small intestine, affecting the pools of hepatic cholesterol and the activities of hepatic regulatory enzymes and LDL receptors. PE and PSY intake resulted in improved plasma cholesterol and TAG concentrations when given in combination with high fat diets while the effects with low fat were less pronounced and limited to decreases in plasma cholesterol.

It is clear from our results that the main secondary response to the action of soluble fiber in the lumen is up-regulation of hepatic apoB/E receptor associated with increased plasma LDL turnover. However, PSY had a more pronounced effect than PE in animals fed high fat due to the specific effects of this soluble fiber on hepatic enzymes of cholesterol synthesis and catabolism as a result of more significant reductions of hepatic cholesterol concentrations by PSY intake.

Manuscript received 15 October 1997, in revised form 26 January 1998, and in re-revised form 24 March 1998.

REFERENCES

1. Krithchevsky, D., and J. A. Story. 1986. Influence of dietary fiber on cholesterol metabolism in experimental animals. *In* Handbook of Dietary Fiber in Human Nutrition. J. A. Spiller, editor. CRC Press, Boca Raton, FL. 129–142.
2. Anderson, J. W., and J. Tietyen-Clark. 1986. Dietary fiber: hyperlipidemia, hypertension and coronary heart disease. *Am. J. Gastroenterol.* **81:** 907–919.
3. Ginsberg, H. N., N-A. Le, J. Melish, D. Steinberg, and W. V. Brown. 1981. Effect of a high carbohydrate diet on apoB catabolism in men. *Metabolism.* **30:** 347–353.
4. Grundy, S. M., and M.A. Denke. 1990. Dietary influence on serum lipids and lipoproteins. *J. Lipid Res.* **31:** 1149–1159.
5. Grundy, S. M. 1986. Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol. *N. Engl. J. Med.* **314:** 745–748.
6. McNamara, D. J. 1992. Dietary fatty acids, lipoproteins and cardiovascular disease. *Adv. Food Nutr. Res.* **36:** 253–351.
7. Fernandez, M. L., K. A. Conde, L. R., Ruiz, C. Montano, J. Ebner, J. and D. J. McNamara. 1995. Carbohydrate type and amount alter intravascular processing and catabolism of plasma lipoproteins in guinea pigs. *Lipids.* **30:** 619–626.
8. Fernandez, M. L., L. Ruiz, A. K. Conde, D-M. Sun, S. K. Erickson, and D. J. McNamara. 1995. Psyllium reduces plasma LDL in guinea pigs by altering hepatic cholesterol homeostasis. *J. Lipid Res.* **36:** 1128–1138.
9. Fernandez, M. L., 1995. Distinct mechanism of plasma LDL lowering by dietary fiber in the guinea pig: specific effects of pectin, guar gum, and psyllium. *J. Lipid Res.* **36:** 2394–2404.
10. Horton, J. D., and D. K. Spady. 1994. Regulation of cholesterol 7α-hydroxylase expression by dietary psyllium in the hamster. *J. Clin. Invest.* **93:** 2084–2092.
11. Everson, G. T., B. O. Daggy, C. Mckinley, and J. Story. 1992. Effects

Downloaded from www.jlr.org by guest, on June 14, 2012

of psyllium hydrophobic mucilloid on LDL-cholesterol and bile acid synthesis in hypercholesterolemic men. *J. Lipid Res.* **33:** 1183–1192.
12. Fernandez, M. L., D-M. Sun, M. A. Tosca, and D. J. McNamara. 1994. Citrus pectin and cholesterol interact to regulate hepatic cholesterol homeostasis and lipoprotein metabolism: a dose response study in guinea pigs. *Am. J. Clin. Nutr.* **65:** 814–822.
13. Fernandez, M.L., M. Vergara-Jimenez, K. Conde, T. Behr, and G. Abdel-Fattah. 1997. Regulation of VLDL-LDL apoB metabolism and plasma lipoprotein processing in guinea pigs by dietary soluble fiber. *Am. J. Clin. Nutr.* **65:** 814–822.
14. Lin, E.C.K., M.L. Fernandez, and D.J. McNamara. 1992. The effects of dietary fat saturation on cholesterol metabolism in the guinea pig. *J. Nutr.* **122:** 2019–2029.
15. Allain, C., L. Poon, C. Chan, W. Richmond, and P. Fu. 1974. Enzymatic determination of total serum cholesterol. *Clin. Chem.* **20:** 470–475.
16. Warnick, G. R., J. Bederson, and J. J. Albers. 1992. Dextran-sulphate-Mg^{+2} precipitation procedure for quantitation of high density lipoprotein cholesterol. *Clin. Chem.* **28:** 1379–1388.
17. Carr, T., C. J. Andressen, and L. L. Rudel. 1993. Enzymatic determination of triglyceride, free cholesterol and total cholesterol in tissue lipid extracts. *Clin. Biochem.* **26:** 39–42.
18. Markwell, M. A. S., S. M. Haas, L. L. Bieber and N. E. Tolbert. 1978. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal. Biochem.* **87:** 206–210.
19. Homquist, L., K. Carlson, and L. A. Carlson. 1987. Comparison between the use of isopropanol and tetramethylurea for the solubilization and quantitation of human serum very low density lipoproteins. *Anal. Biochem. J.* **149:** 423–426.
20. Chapman, J. M., G. L. Mills, and J. H. Ledford. 1975. The distribution and partial characterization of the serum apolipoproteins in the guinea pig. *Biochem. J.* **149:** 423–436.
21. Stoffel, W., and T. Demant. 1981. Selective removal of apoB-containing serum lipoproteins from blood plasma. *Proc. Natl. Acad. Sci. USA.* **12:** 611–615.
22. Ishida, B.Y., and B. Paigen. 1992. Silver-enhanced radial immunodiffusion assay of plasma lipoproteins. *J. Lipid. Res.* **33:** 1073–1077.
23. Shapiro. D. L., R. L. Imblum, and V. W. Rodwell. 1969. Thin-layer chromatographic assay for HMG-CoA reductase and mevalonic acid. *Anal. Biochem.* **31:** 383–390.
24. Smith, J. L., J. Jersey, S. P. Pillar, and I. R. Hardie. 1986. Hepatic acyl-CoA cholesterol acyltransferase. Development of a standard assay and determination in patients with cholesterol gallstones. *Clin. Chem. Acta.* **158:** 271–282.
25. Shefer, S., S. Hauser, and E. H. Mosbach. 1968. 7α-Hydroxylation of cholesterol by rat liver microsomes. *J. Lipid Res.* **9:** 328–333.
26. Jelinek, D. F., D. Andersson, C. A. Slaughter, and D. D. Russell. 1990. Cloning and regulation of cholesterol 7α-hydroxylase, the rate limiting enzyme in the bile acids biosynthesis. *J. Biol. Chem.* **265:** 8190–8197.
27. Goldstein, J. L., S. K. Basu, and M. S. Brown. 1983. Receptor-mediated endocytosis of low density lipoprotein in cultured cells. *Methods. Enzymol.* **98:** 241–260.
28. Keightley, D. D., R. J. Fischer, and N. A. C. Cressie. 1983. Properties and interpretation of the Woolf and Scatchard plots in analyzing the data from steroid receptor assays. *J. Steroid Biochem.* **9:** 1407–1412.
29. Abdel-Fattah, G., M. L. Fernandez, and D. J. McNamara. 1995. Regulation of guinea pig very low density lipoprotein secretion rates by dietary fat saturation. *J. Lipid. Res.* **36:** 1188–1198.
30. Fernandez, M. L., G. Abdel-Fattah and D. J. McNamara. 1995. Differential effects of simple vs. complex carbohydrates on VLDL secretion rates and HDL metabolism in the guinea pig. *Biochim. Biophys. Acta.* **1256:** 31–38.
31. National Research Council. 1978. Nutrient Requirement of Laboratory Animals. National Academy of Sciences, Washington, D.C.
32. Egusa, G., D. W. Brady, S. M. Grundy, and B. V. Howard. 1987. Isopropanol precipitation method for the determination of apolipoprotein B specific activity and plasma concentrations during metabolic studies of very low density lipoprotein and low density lipoprotein apoprotein B. *J. Lipid Res.* **24:** 1418–1428.
33. Matthews, C. M. E. 1957. The theory of tracer experiments with ^{131}I-labeled plasma proteins. *Phy. Med. Biol.* **2:** 36–53.
34. Abbot, W. G. H., B. Swinburn, G. Rutuolo, H. Hara, L. Patti, L. Harper, S. M. Grundy, and B. V. Howard. 1990. Effect of a high-carbohydrate low-saturated fat diet on apolipoprotein B and triglyceride metabolism in Pima Indians. *J. Clin. Invest.* **86:** 642–650.
35. Sprecher, D. L., B. V. Harria, A. C. Goldberg, E. C. Anderson, L. M. Mayuk, B. S. Russell, D. S. Crone, C. Quimm, J. Bateman, B. R. Kuzmak, and L. D. Allgood. 1993. Efficacy of psyllium in reducing serum cholesterol levels in hypercholesterolemic patients on high- or low-fat diets. *Ann. Intern. Med.* **7:** 545–554.
36. Berglund, L., M. F. Sharkey, R. L. Elam, and J. L. Witztum. 1989. Effects of lovastatin therapy on guinea pig low density lipoprotein composition and metabolism. *J. Lipid Res.* **30:** 1591–1600.
37. West, C. E., D. R. Sullivan, and M. B. Katan. 1990. Boys from populations with high carbohydrate intake have higher fasting triglycerides levels than boys from populations with high-fat intake. *Am. J. Epidemiol.* **131:** 271–282.
38. Huff, M. W., D. E. Telford, P. H. R. Barrett, J. T. Billheimer and D. J. Gillies. 1994. Inhibition of hepatic ACAT decreases apoB secretion in miniature pigs fed cholesterol-free diet. *Arterioscler. Thromb.* **14:** 1498–1508.
39. Fernandez, M. L., E. C. K. Lin, and D. J. McNamara. 1992. Regulation of guinea pig plasma low density lipoprotein kinetics by dietary fat saturation. *J. Lipid Res.* **33:** 97–109.
40. Ebihara, K., and B. O. Schneeman. 1989. Interaction of bile acids, phospholipids, cholesterol and triglyceride with dietary fibers in the small intestine in rats. *J. Nutr.* **119:** 1100–1106.
41. Vuorinen-Markkola, H., M. Sinisalo, and V. A. Kovisto. 1992. Guar gum in insulin-dependent diabetes: effects on glycemic control and serum lipoproteins. *Am. J. Clin. Nutr.* **56:** 1056–1060.
42. Romero, L., and M. L. Fernandez. 1996. Dietary fat amount and carbohydrate type regulate hepatic acyl-CoA:cholesterol acyltransferase (ACAT) activity. Possible links between ACAT activity and plasma cholesterol levels. *Nutr. Res.* **16:** 937–948.
43. Suckling, K. E., and E. F. Stange. 1985. Role of acyl-CoA:cholesterol acyltransferase in cellular cholesterol metabolism. *J. Lipid Res.* **26:** 647–670.
44. Melish, J., N-A. Le, H. Ginsberg, D. Steinberg, and V. Brown. 1980. Dissociation of apoprotein B and triglyceride production in very low density lipoproteins. *J. Am. Physiol.* **239:** E354–E362.
45. Elovson, J., J. E. Chatterton, G. T. Bell, V. N. Schumaker, M. A. Reuben, D. L. Puppione, J. R. Reeve, Jr., and N. L. Young. 1988. Plasma very low density lipoproteins contain a single molecule of apolipoprotein B. *J. Lipid Res.* **29:** 1461–1473.
46. Ginsberg, H. N. 1990. Lipoprotein physiology and its relationship to atherogenesis. *Endocrinol. Metab. Clin. North Am.* ***19:*** 211–22.
47. Mazur, A., C. Remsey, E. Gueux. M-A. Levrat, and C. Demigne. 1990. Effects of diets rich in fermentable carbohydrates on plasma lipoprotein levels and lipoprotein catabolism in rats. *J. Nutr.* **120:** 1037–1045.
48. Turley, S. D., and J. M. Dietschy. 1995. Mechanisms of LDL-cholesterol lowering action of psyllium hydrophyllic mucilloid in the hamster. *Biochim. Biophys. Acta.* **1255:** 177–184.

Downloaded from www.jlr.org by guest, on June 14, 2012