Native and Partially Hydrolyzed Psyllium Have Comparable Effects on Cholesterol Metabolism in Rats

Ba ham H. Arjmandi, Eugenia Sohn, Shanil Juma, Shreedhar R. Murthy* and Bruce P. Daggy*

University of Illinois at Chicago, Chicago, IL 60612 and *The Procter & Gamble Company, Mason, OH 45040

ABSTRACT This study was conducted to determine whether the storage conditions and the levels of psyllium in the diet modulate its hypocholesterolemic effects. Seventy-five male Sprague-Dawley rats, age 90 d, were randomly divided into five treatment groups and were fed cholesterol-containing diets for 21 d. Diets included 10% cellulose (control); 5 or 10% psyllium stored 8 mo at 5°C (PS5); or 5 or 10% psyllium stored 8 mo at 40°C (PS40). The higher storage temperature caused a gradual decrease in molecular weight of the psyllium, as measured by changes in solution viscosity. Hepatic rates of sterol synthesis were significantly (P < 0.001) higher in all of the psyllium-fed rats compared with control rats [21 ± 2, 312 ± 35, 464 ± 40, 328 ± 49 and 439 ± 57 nmol [3H]digitonin-precipitable sterol (DPS)/g liver-h], respectively, for control, 5% PS5, 10% PS5, 5% PS40 and 10% PS40]. A similar trend was observed in intestinal rates of sterol synthesis, and the difference was significant (P < 0.05) for all treatment groups except the 5% PS5-fed group compared with the control group. Liver total cholesterol and total lipid concentrations were significantly lower in all psyllium-fed rats compared with controls. There were no significant differences in serum total cholesterol concentrations among the psyllium-fed groups, although serum cholesterol levels in both the PS5-fed groups were significantly (P < 0.05) lower than in the control group (2.66 ± 0.18, 2.62 ± 0.15 and 3.26 ± 0.12 mmol/L, respectively, for 5% PS5, 10% PS5 and control). Serum triglyceride and HDL cholesterol concentrations did not vary significantly among groups. The findings of this study indicate that the cholesterol-lowering activity of psyllium is unaltered by storage conditions shown to cause a moderate degree of hydrolysis.


KEY WORDS: • sterol synthesis • viscosity • dietary fiber • liver • intestine • rats

Elevated blood cholesterol has been implicated as a major risk factor for coronary heart disease (CHD) (Kannel et al. 1971). There is a general consensus that reducing blood cholesterol levels will lower the incidence of CHD. There has been much interest in the utilization of soluble fiber to lower total blood cholesterol. Findings of various studies suggest that the incorporation of certain soluble fibers into the diets of humans and animals can lower blood cholesterol, particularly LDL cholesterol (Truswell and Beynen 1992).

One such soluble fiber is psyllium husk fiber. Clinical studies have shown that fiber supplements or foods providing about 10 g psyllium/d reduce LDL cholesterol by ~5–9% below the levels achieved with a prudent diet, American Heart Association (AHA) Step I, (e.g., Bell et al. 1989, Levin et al. 1990, Sprecher et al. 1993, Stoy et al. 1993, Wolever et al. 1994), without altering HDL cholesterol or serum triglyceride concentrations. Studies in both humans (Everson et al. 1992, Miettinen and Tarpila 1989) and animals (Arjmandi et al. 1992a, Horton et al. 1994, Matheson and Story 1995, Turley et al. 1996) suggest that the primary mechanism by which dietary soluble fiber lowers cholesterol is through enhanced synthesis of bile acids and their fecal excretion. The enhanced elimination of bile acids also results in increased hepatic cholesterol synthesis (Arjmandi et al. 1992b, Horton et al. 1994, Turley et al. 1991).

There are a limited number of studies which have examined the effects of processing on certain soluble fibers and their cholesterol-lowering capacities. In a recent study, Lia et al. (1995) investigated the effects of β-glucan with and without enzymatic degradation on the excretion of bile acids from the small intestine of humans. The results of the study supported the hypothesis that higher molecular weight β-glucan is more effective in increasing bile acid excretion than the lower molecular weight β-glucan and hence implied a greater effectiveness at lowering serum lipid levels. Gallaher et al. (1993) studied the relationship between the viscosity and fermentability of dietary fiber on cholesterol and bile acid metabolism using a synthetic fiber, hydroxypropylmethyl cellulose (HPMC), and guar gum in a hamster model. They showed that high molecular weight, high viscosity HPMC was more potent for maintaining low plasma and hepatic cholesterol levels than a low molecular weight, low viscosity form. The investigators also observed that guar gum tended to be more effective in increasing bile acid excretion than the lower molecular weight β-glucan.
effective in lowering cholesterol in its native form than in its hydrolyzed form. Similarly, in a clinical trial by Superko et al. (1988), it was shown that consumption of guar gum with high viscosity was somewhat more effective in suppressing plasma total and LDL cholesterol compared with medium viscosity guar gum. The relationship between the hypocholesterolemic effects of psyllium and its molecular weight requires examination.

The objectives of this study were twofold: 1) to determine whether storage of psyllium hydrophilic mucilloid at higher temperature (40°C vs. 5°C) would alter its viscosity, which ultimately may modulate its hypocholesterolemic effects; and 2) to compare the hypocholesterolemic effects of 5 and 10% psyllium held in the aforementioned storage conditions, with a fixed background of dietary lipids, protein and total dietary fiber.

MATERIALS AND METHODS

Preparation and characterization of psyllium. Psyllium husk USP (Plantago ovata, sourced from India) was commercially sanitized, milled, and formulated into psyllium hydrophilic mucilloid USP (Sugar Free Orange Smooth Texture Metamucil®, The Procter & Gamble Co., Cincinnati, OH). The husk used was 95% pure by pharmacopeial methods (USP 1995) and contained ~8% moisture.

The finished product contained 3.4 g psyllium husk per 3.8 g product. Excipients in the product included maltodextrin, citric acid, aspartame, flavors and dyes. The product was stored in sealed high density polyethylene canisters at either 5°C (PS1) or 40°C (PS4) for 8 mo. During this period, samples were drawn at 0, 1, 2, 3, 6 and 8 mo for analysis of specific viscosity and swell volume as described below.

Specific viscosity determination. A viscosity measurement was used to determine hydrolysis occurring over time, which was observed under the high-temperature storage condition. The average molecular weight (Mw) of a polymer can be measured by solution viscometry. Intrinsic viscosity [η], defined as reduced viscosity at infinite dilution, is related to the average Mw via the Mark-Houwink equation, [η] = K Mwα, where K and α are constants for a given combination of polymer and solvent (Beychok 1971). It is determined by plotting reduced viscosity (specific viscosity divided by the concentration of polymer in solution) as a function of varying concentrations. The y-intercept is the intrinsic viscosity, usually expressed in deciliters per gram. For measuring relative changes in the Mw, when K and α are unknown, the specific viscosity (SV) of a set of sample solutions at a fixed concentration can be used. Specific viscosity is defined as SV = (t/t0 - 1), where t and t0 are the times (usually in seconds) to flow through a capillary viscometer for the test and solvent solutions, respectively.

An important requirement to measure the Mw or SV of a polymer by viscometry is the availability of a suitable solvent. Guanidine hydrochloride at a concentration of 4–8 mol/L has been used to dissolve proteins or proteoglycans for measuring their molecular weights either by viscometry (Kitchen and Cleland 1978) or gel permeation chromatography (Ui 1981). We found that the psyllium polysaccharide readily dissolves in 4 mol/L guanidine hydrochloride. Excipients in the product were found not to contribute to the viscosity of the solution (data are not shown). This medium was used for solution viscometric studies of psyllium-containing products.

The specific viscosity of solutions of the PS1 and PS4 at the same concentrations (3.2 g/L) was measured in duplicate at each time point in 4 mol/L guanidine hydrochloride using a Cannon-Ubbelohde (Baxter Healthcare, Deerfield, IL) viscometer. Any change in specific viscosity was attributed to changes in the molecular weight of the psyllium polysaccharide.

Swell volume determination. Swell volume is a standard test (USP 1995) used to assess the quality of psyllium husk and psyllium hydrophilic mucilloid. The standard test was modified to improve sensitivity and reproducibility. Briefly, product equivalent to 0.5 g psyllium husk was added to a 100-mL graduated cylinder and brought to volume with distilled water. The cylinder was capped and inverted several times until a uniform suspension was achieved. The inversion step was repeated at 4 and 8 h from the start of the test, and the swell volume was read at 24 h. The average of the duplicate determinations is reported. In the standard test, 3.5 g of psyllium husk held in its native form than in its hydrolyzed form.

Approximateness of rat model. To conduct the study, cholesterol-fed rats were used. The rat model was chosen over the commonly used hamster model, because of a concern raised by other investigators (Gallagher et al. 1993) that fermentation in the hamster’s forestomach may diminish the differences in fiber viscosity.

Animals and diets. Seventy-five male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN), age 90 d, were acclimated to a standard laboratory nonpurified diet (Teklad diet #8640, Teklad, Madison, WI) for at least 4 d. Using a randomized complete block design, rats were divided by initial body weight into five groups of 15 rats each. Three animals from each of the five treatments were included in every block. Rats were housed individually in an environmentally controlled animal laboratory with a 12-h light/dark cycle. The room was illuminated from 1400 to 0200 h. For 3 wk, rats were freely fed one of the five diets (Table 1) with unlimited access to water. Diets contained 10% cellulose (control); 5 or 10% psyllium stored at 4°C (PS1); or 5 or 10% psyllium stored at 40°C (PS4) for 8 mo. Diets were formulated to be isocaloric and isonitrogenous, and adjustments were made to account for Metamucil excipients.

Animal necropsy and processing of samples. After 21 d of treatment, at the midpoint of the dark cycle and within a 1-h period, rats were slightly anesthetized (mixture of ketamine:xylazine, 100:5 ratio, respectively) and were rapidly (~10 s) injected with 1.85 GBq of tritiated water ([3H]water) (Amersham, Arlington Heights, IL) in 0.5 mL of isotonic NaCl into the femoral artery of each rat. This time was considered zero time and 4-min intervals were allowed between injections. Sixty minutes later, the animals were anesthetized with ketamine:xylazine mixture, and blood was collected from the abdominal aorta for measuring serum water activity and lipid and cholesterol analyses. Serum was separated by centrifugation at 1500 × g for 20 min at 4°C. Aliquots of serum were frozen and kept at −20°C for later analyses. The liver was immediately removed, rinsed with ice-cold 0.154 mol/L NaCl solution, and processed for determining the rates of [3H]water incorporation into digitonin-precipitable sterols (DPS). Portions of the livers were also kept in sealed containers and stored at −70°C for lipid and cholesterol analyses. Similarly, the

### Table 1

**Composition of diets**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Cellulose</th>
<th>5% Psyllium</th>
<th>10% Psyllium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/100 g</td>
<td>g/100 g</td>
<td>g/100 g</td>
</tr>
<tr>
<td>Sucrose</td>
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<td>10</td>
</tr>
<tr>
<td>Com starch</td>
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<td>36</td>
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<td>20</td>
<td>20</td>
</tr>
<tr>
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<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Hydrogenated coconut oil</td>
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<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Cellulose</td>
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</tr>
<tr>
<td>Metamucil</td>
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<td>17.2</td>
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<tr>
<td>Metamucil excipients</td>
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<td>3.6</td>
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</tr>
<tr>
<td>DL-Methionine</td>
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<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
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<tr>
<td>Choline bitartrate</td>
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<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Vitamin mixture</td>
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<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

1 The diets were prepared by E. A. Ulman, Research Diets, New Brunswick, NJ.
2 Aucile PH-101® microcrystalline cellulose (FMC, Philadelphia, PA).
3 Sugar-Free Orange Smooth Texture Metamucil (Procter & Gamble Co., Cincinnati, OH); 58 g/100 g psyllium husk fiber.
4 Metamucil excipients include maltodextrin, citric acid, aspartame, flavors and dyes; provided by Procter & Gamble Co.
5 AIN-76 mineral and vitamin mixtures were from ICN Biochemicals (Costa Mesa, CA).
entire small intestine was removed, freed from any adhering tissues. The intestinal contents were immediately flushed out using isotonic ice-cold sodium chloride solution. The intestines were blotted with cotton gauze, and weighed before measuring the rate of sterol synthesis.

In vivo intestinal and hepatic rates of sterol biosynthesis. The rates of sterol synthesis were assessed in intestinal and hepatic tissues as the primary end points because these rates are more responsive to treatments than plasma cholesterol in this animal model (Arjmandi et al. 1992b, Turley et al. 1991), making sterol synthesis rate an appropriate measure to detect small changes in fiber potency. A modified method of Dietschy and Siperstein (1965) was used to assess the in vivo hepatic and intestinal rates of sterol synthesis. The modified method has been described elsewhere (Arjmandi et al. 1992b). The specific activity of serum water used to calculate the rates of tritiated water incorporation into DPS was determined according to the following formula (Jeske and Dietschy 1980):

\[
(kBq \text{ H/L serum}) \times ([(1.09)/\mu\text{mol water/L water}])
\]

The small intestine was placed in a 100-mL beaker containing 80 mL 5% alcoholic KOH and saponified on a steam bath with occasional stirring until the tissue was completely dissolved. The dissolved tissue was then brought to a volume of 50 mL using 100% ethanol. Three 10-mL aliquots were then placed in glass tubes with teflon caps (25 × 150 mm) and determined to determine rates of \(^{[3}H\)water incorporation into DPS. Aliquots of liver in triplicate ranging from 500 to 800 mg were placed in similar glass tubes with teflon caps and saponified in 50% alcoholic KOH in a water bath for 2–3 h. Sterols were extracted twice with 30 mL petroleum ether, and the pooled ether extracts were gently evaporated to dryness under a fume hood. The residue was dissolved in 3 × 2 mL ethanol:acetone (1:1) and transferred into centrifuge tubes. The contents of the tube were acidified with a drop of 1 mol/L HCl, and sterols were precipitated with 2 mL of 0.5% digitonin in 5% ethanol. The digitonin precipitate was washed twice with 5 mL acetone, followed by 3 mL diethyl ether. The precipitate was air dried, followed by an additional 15 min under vacuum at 80°C. The digitonin precipitates were then dissolved in pyridine, and the free sterols were extracted using 3 mL diethyl ether, transferred to a counting vial and dried. The air-dried free sterol extracts were further dried in a vacuum oven at 80°C for 1 h. The extract was dissolved in 1 mL methanol, scintillation solution (Scintiverse, BOA, FisherBiotech, Fair Lawn, NJ) was added and the mixture was analyzed for \(^{[3}H\)radioactivity using a liquid scintillation analyzer. The rate of \(^{[3}H\) incorporated into DPS was calculated as nanomoles of \(^{[3}H\)water per hour per gram of tissue or whole organ weight.

Serum and liver lipid analyses. Serum total cholesterol and triacylglyceride (TG) were measured using enzymatic kits (Sigma Diagnostics, St. Louis, MO). Serum HDL cholesterol was determined by precipitation technique (Sjoberlom and Eklund 1989). Portions of liver were homogenized, then extracted with a 2:1 (v/v) chloroform:methanol mixture. After addition of 0.13 mol/L NaCl solution to the extraction and separation of phases, aliquots of the organic phase were analyzed for liver total cholesterol. Liver total cholesterol was determined using a color reagent of glacial acetic acid-FeSO\(_4\)-H\(_2\)SO\(_4\) (Searcy and Bergquist 1960). Total liver lipids were determined using the Folch gravimetric method (Folch et al. 1957). Briefly, the remainder of the organic phase was evaporated, dried to constant weight and weighed to measure total lipids.

Statistical analyses. Data analysis (GraphPad Instat Software version 2.00) involved estimation of means and standard error of means for each of the groups (Snedecor and Cochran 1967). ANOVA was performed to determine whether there were significant (P < 0.05) differences among the groups. When an ANOVA indicated any significant difference among the means, the Tukey-Kramer follow-up multiple comparison test was used to determine which means were significantly different (Chew 1977). When Bartlett’s test indicated that heterogeneity of variances existed, a log transformation was used to achieve equal variances, and the ANOVA and Tukey-Kramer tests were performed on the transformed data. Unless otherwise stated, results are expressed as means ± SEM.

RESULTS

Psyllium viscosity. Samples stored for 8 mo at 5 or 40°C had average specific viscosities of 1.23 and 0.83, respectively (Fig. 1). PS\(_{50}\) exhibited a progressive decline in SV and appeared linear over time [first order, least squares regression: SV = -0.0466(month) + 1.2044; \(r^2 = 0.96\)]. PS\(_3\) showed no change in SV from base line (slope of regression line = 0.0025, \(r^2 = 0.25\)). SV correlated with swell volume for PS\(_{50}\) samples [Fig. 2; Swell Volume = 15.31(SV) + 16.10; \(r^2 = 0.92\)].

Food and water intake. Food and water intakes of rats were not significantly affected by any of the treatments. The mean food consumption among the treatment groups ranged from 18.4 to 20.5 g/d and the mean water intake ranged from 29.2 to 30.5 mL/d.

Body and organ weights. The five treatment groups started with similar mean body weights ranging from 336 to 337 g. All rats gained weight during the study period with no differences across treatments in mean final body weights (411 ± 3.7, 411 ± 4.0, 412 ± 4.1, 406 ± 4.8, and 416 ± 3.1 g for 10% CE, 5%PS\(_3\), 10% PS\(_5\), 5%PS\(_{50}\) and 10% PS\(_{50}\) groups, respectively). Relative liver weights (g/100 g body weight) for all psyllium-fed groups, irrespective of psyllium dose or storage condition were significantly (P < 0.05) lower than the 10% cellulose-fed group (Table 2). In contrast, the relative small intestine weights of all psyllium-fed groups were significantly greater than that of the 10% cellulose-fed controls.

Serum total cholesterol, lipoprotein cholesterol and serum total triglycerides. The effects of dietary treatments on serum total and HDL cholesterol and serum triglyceride are presented in Table 3. Serum total cholesterol levels tended to be lower in all of the psyllium-fed rats compared with the 10% cellulose-fed controls. However, serum total cholesterol concentrations were significantly lower than controls only in those rats fed either 5 or 10% PS\(_3\). Serum HDL and TG levels were not significantly affected by psyllium feeding (Table 3). Serum lipid concentrations did not differ among the four psyllium-fed groups.
Hepatic lipids, cholesterol and sterol synthesis. Groups that received psyllium had significantly lower liver total lipids and total cholesterol compared with control rats (Table 3). Psyllium feeding at both 5 and 10% and at either viscosity significantly (P < 0.05) increased the rates of hepatic sterol biosynthesis (Fig. 3A). When hepatic rates of sterol synthesis were expressed in terms of the whole liver, results were similar (Fig. 3B).

Small intestinal sterol synthesis. The intestinal rates of sterol biosynthesis were also greater in rats fed psyllium. When the rates were expressed per gram of small intestine, the differences were significant (P < 0.05) in all groups except those fed 5% PS5 (Fig. 3C). When the rates of sterol synthesis were expressed in terms of the whole small intestine, the differences were significant (P < 0.05) in all of the psyllium-fed groups compared with the cellulose-fed controls (Fig. 3D).

TABLE 2
Effects of native and partially hydrolyzed psyllium on organ weights in rats

<table>
<thead>
<tr>
<th>Dietary treatment</th>
<th>Liver weight</th>
<th>Intestine weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/100 body weight</td>
<td>g/100 g body wt</td>
</tr>
<tr>
<td>10% CE2</td>
<td>15.2 ± 0.3a</td>
<td>3.7 ± 0.1a</td>
</tr>
<tr>
<td>5% PS5</td>
<td>14.1 ± 0.1ab</td>
<td>3.4 ± 0.1b</td>
</tr>
<tr>
<td>10% PS5</td>
<td>13.6 ± 0.3b</td>
<td>3.3 ± 0.1b</td>
</tr>
<tr>
<td>5% PS40</td>
<td>13.7 ± 0.4b</td>
<td>3.4 ± 0.1b</td>
</tr>
<tr>
<td>10% PS40</td>
<td>13.8 ± 0.3b</td>
<td>3.3 ± 0.1b</td>
</tr>
</tbody>
</table>

1 Values reported are means ± SEM, n = 14–15. Within each column, values with the same superscript are not significantly different (P < 0.05).
2 CE = cellulose; PS5 = psyllium stored at 5°C; PS40 = psyllium stored at 40°C.

DISCUSSION

The viscosity and the gel-forming ability of dietary fiber have been suggested in a limited number of studies to play important roles in lowering blood cholesterol (Gallagher et al. 1993, Superko et al. 1988) and improving glycemic control (Edwards et al. 1987, Jenkins et al. 1978). Because of its viscosity, dietary fiber is believed to interfere with cholesterol and/or bile acid absorption (Carr et al. 1996, Eastwood and Morris 1992), thereby reducing blood cholesterol levels. The hypocholesterolemic action of a given fiber might be predicted based on its ability to form gel in vitro before ingestion. The concerns are at least twofold: 1) that factors such as heat, acid and specific nonmammalian enzymes can cause hydrolysis of dietary fibers and hence result in reduced viscosity and capability to form firm gels and 2) that the expression of viscous dietary fiber to the gastric environment and juices may substantially lower the original viscosity measured in vitro (Edwards et al. 1987), thereby making the in vitro viscosity and gel-forming ability of the polysaccharides less meaningful. In spite of these limitations, reporting the in vitro viscosity might at a minimum allow more confident comparisons of results across studies.

Although we are not aware of any studies in which the relationship between psyllium’s viscosity and its cholesterol-lowering functionality has been reported, it is reasonable to assume that incomplete hydrolysis of psyllium could result in loss of its hypocholesterolemic effect despite the analytical standards of known molecular weight limited our ability to convert the change in viscosity to an absolute change in molecular weight. However, the well-understood conditions for hydrolysis to occur, combined with the absence of excipient effects on viscosity and the correlation of SV to swell volume, leave no doubt that hydrolysis of the psyllium was indeed taking place.

The robust activity of the psyllium in this study may be due in part to its initial quality, which, although typical of the quality used in the commercial product, exceeded USP standards for purity (95% pure husk vs. 85% minimum requirement) and swell volume (about 70 mL gel/g psyllium in finished product vs. ~31.4 mL/g minimum). It should be noted that the product still met or exceeded all USP tests for psyllium hydrophilic muciloid after 8 mo storage at 40°C. However, the overwhelming majority of clinical trials in which psyllium was fed 5% PS40 (Fig. 3A).

FIGURE 2 Swell volume of psyllium as a function of specific viscosity during prolonged storage at 40°C. The psyllium was formulated into a dry powdered beverage mix and stored in high density polypropylene canisters. The specific viscosity of solutions of the samples at a fixed concentration (0.32%) were measured in duplicate at each time point in 4 mol/L guanidine hydrochloride using a Cannon-Ubbelohde viscometer. To measure swell volume, product equivalent to 0.5 g psyllium husk was added to a 100-mL graduated cylinder and was brought to volume with distilled water. The cylinder was capped and inverted several times until a uniform suspension was achieved. The inversion step was repeated at 4 and 8 h from the start of the test, and the swell volume was read at 24 h. The mean of duplicate determinations is the 6-mo time point was deleted as an outlier for swell volume.

The loss of its hypocholesterolemic effect despite the analytical standards for purity (95% pure husk vs. 85% minimum requirement) and swell volume (about 70 mL gel/g psyllium in finished product vs. ~31.4 mL/g minimum). It should be noted that the product still met or exceeded all USP tests for psyllium hydrophilic muciloid after 8 mo storage at 40°C. However, the overwhelming majority of clinical trials in which psyllium was fed 5% PS40 (Fig. 3A).

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TABLE 3

Effects of native and partially hydrolyzed psyllium on liver total lipids and cholesterol, serum triglyceride, and total and HDL cholesterol in rats

<table>
<thead>
<tr>
<th>Dietary treatment</th>
<th>Liver total lipids</th>
<th>Liver total cholesterol</th>
<th>Serum total cholesterol</th>
<th>Serum HDL cholesterol</th>
<th>Serum triglyceride</th>
</tr>
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<td>mg/g</td>
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<tr>
<td>10% CE2</td>
<td>38.4 ± 0.96a</td>
<td>33.6 ± 1.53a</td>
<td>3.26 ± 0.12a</td>
<td>1.66 ± 0.14</td>
<td>2.07 ± 0.23</td>
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<td>5% PS5</td>
<td>31.6 ± 0.53b</td>
<td>17.3 ± 1.29b</td>
<td>2.66 ± 0.18b</td>
<td>1.62 ± 0.10</td>
<td>2.33 ± 0.25</td>
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<tr>
<td>10% PS5</td>
<td>30.1 ± 0.69b</td>
<td>14.7 ± 0.96b</td>
<td>2.62 ± 0.15b</td>
<td>1.96 ± 0.18</td>
<td>2.57 ± 0.27</td>
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<tr>
<td>5% PS40</td>
<td>31.9 ± 0.66b</td>
<td>15.8 ± 0.83b</td>
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<td>1.75 ± 0.09</td>
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<td>10% PS40</td>
<td>31.2 ± 0.67b</td>
<td>15.0 ± 1.19b</td>
<td>2.82 ± 0.10ab</td>
<td>1.70 ± 0.07</td>
<td>2.13 ± 0.37</td>
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</table>

1 Values reported are means ± SEM, n = 14–15. Within each column, values with the same superscript are not significantly different (P < 0.05).

2 CE = cellulose; PS5 = psyllium stored at 5°C; PS40 = psyllium stored at 40°C.

was evaluated for hypocholesterolemic activity have reported cholesterol-lowering effects (Anderson et al. 1990). This suggests that the hypocholesterolemic property of psyllium is generally present and preserved despite variations in husk quality and current practice in formulation, processing and storage conditions. The results of this study cannot predict the sustained effectiveness of lower grades of psyllium or psyllium which has been subjected to harsher processing or storage conditions. At this point, given the lack of reference standards for psyllium, clinical trials remain the ultimate proof of an efficacious product.

The findings of this study confirm that feeding 5 or 10% psyllium can profoundly alter cholesterol metabolism in rats, particularly as judged by observing the sensitive measure of hepatic sterol synthesis. In this study, significant alterations in cholesterol metabolism were achieved by either of the psyllium doses, suggesting that a threshold level for the effectiveness of psyllium has to be defined. Several clinical trials (e.g., Ander-

**FIGURE 3** Effect of psyllium on in vivo rates of hepatic and intestinal sterol syntheses. The data are expressed as nanomoles of [3H] water incorporated into digitonin-precipitable sterols (DPS) per gram of liver and small intestine (A, C) or whole liver and whole small intestine (B, D). Bars represent mean ± SEM; n = 14–15 rats. Bars which do not share the same letters are significantly (P < 0.05) different. CE = cellulose; PS5 = psyllium stored at 5°C; PS40 = psyllium stored at 40°C.
son et al 1988, Bell et al. 1989, Levin et al. 1990, Sprecher et al. 1993, Stoy et al. 1993, Wolfever et al. 1994) indicate that doses of psyllium ranging from 3.6 to 24.2 g on a daily basis lower blood cholesterol by 5–20%. Based on these studies, the dose-response curve in humans does not appear to be steep. Doses of ~10 g psyllium husk/d have been tested repeatedly and found to be effective. A number of animal studies have used diet formulations containing 5–10% psyllium. Our study was conducted in the context of this body of work. Psyllium was well tolerated by rats at the level of 5–10%, as judged by visual observation of the animals, food intake, weight gain and appearance of the organs. This signifies that even large doses of psyllium may not produce deleterious effects, at least in the model studied.

There are a number of proposed mechanisms by which psyllium and other soluble fibers may lower serum cholesterol. Among these mechanisms, suppression of hepatic sterol synthesis has been suggested (Anderson et al. 1990). Based on our findings in the present study and our earlier observations (Arjmandi et al. 1992b) as well as the data from other investigators (Horton et al. 1994, Turley et al. 1991), it is reasonable to rule out this mechanism for psyllium’s effects. In contrast to this proposed mechanism, psyllium and some of the other soluble fibers (Illman and Topping 1985) actually enhance the hepatic and intestinal rates of sterol synthesis in order to compensate for the fecal loss of sterols and bile acids. The fecal loss of sterols must be greater than the enhanced compensatory synthesis of sterols by these organs, resulting in a net sterol loss which is reflected in lower liver and serum cholesterol concentrations.

The relative intestinal weights of psyllium-fed rats irrespective of the dose or viscosity were significantly higher than those of cellulose-fed controls. The higher intestinal weights in psyllium-fed rats may be due in part to enhanced cell proliferation. Marsman and McBurney (1996) have reported that in vitro incorporation of certain soluble fibers increased cell proliferation in isolated colonocytes. The investigators did not find any relation between the presence of short-chain fatty acids and increased proliferation of colonocytes, despite the known effect of short-chain fatty acids on mucosal development and epithelial cell division (Roediger 1980). In vivo studies have also reported that certain soluble fibers such as psyllium and oat bran increase intestinal weight. Schneeman and Richter (1993) measured the weights of the cecum and small intestine in rats between the ages of 3.5 and 18.5 mo and found that the increases were significantly greater in those fed psyllium compared with the control rats. The authors speculated that these differences, at least in the case of ileal weights, were due to the exposure of intestinal cells to more nutrients.

In conclusion, our data indicate that psyllium that has been partially hydrolyzed as a result of storage conditions exerts effects on cholesterol metabolism similar to that of its native form. Additional studies are required to further define the relationship between the dose and viscosity of soluble fibers and their cholesterol-lowering activity.

LITERATURE CITED

