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ORIGINAL CONTRIBUTION

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The hypocholesterolemic effect of lemon peels, lemon pectin, and the waste stream material of lemon peels in hybrid F_1B hamsters

Summary *Background* We found in preliminary studies with hamsters that citrus peels have a cholesterol lowering effect comparable to that of pectin extracted from these peels. *Aim of the study* We wanted to examine whether the cholesterol lowering effect of the peels could be completely accounted for by the pectin in the peels. *Methods* We fed cholesterol enriched (0.1 %,w/w) semipurified diets containing 3 % (w/w) of cellulose, lemon peels, lemon pectin, and the waste stream material of the lemon peels to hybrid F_1B hamsters for a period of 8 weeks. The waste stream of the lemon peels is the left over after extraction of the lemon pectin. *Results* Feeding the semipurified diets resulted in an increase of plasma cholesterol levels in all the dietary groups after 2 and 4 weeks on the diets. Cholesterol concentrations in the cellulose fed hamsters continued to increase after 4 weeks on the diet, whereas cholesterol levels in the other groups had reached a plateau. As a consequence, the plasma cholesterol levels in the hamsters fed the peels (5.59 ± 0.74 mmol/L, mean ± SD, n = 14), pectin (5.19 ± 0.48 mmol/L), or waste stream (5.53 ± 0.94 mmol/L) were lower than those in the hamsters fed cellulose (6.71 ± 1.52 mmol/L) after 8 weeks on the diets. Differences in total plasma cholesterol were reflected in differences in both VLDL and LDL cholesterol concentration, but this effect was more distinct for the VLDL. There was no effect of the type of fiber on HDL cholesterol levels. Liver cholesterol concentrations paralleled the concentrations of plasma cholesterol and the liver cholesterol concentrations in the hamsters fed the peels (3.57 ± 1.01 µmol/g liver, mean ± SD, n = 14), pectin (4.86 ± 1.42), and the waste stream (4.96 ± 1.89) were lower than those in the cellulose group (7.19 ± 2.32). The hamsters fed the peels, pectin, or waste stream tended to have a higher excretion of fecal bile acids and neutral sterols then the cellulose fed hamsters. *Conclusion* The results of this study suggest that lemon peels and the waste stream of the lemon peels are as effective in lowering plasma and liver cholesterol in hamsters as the pectin extracted from the peels and that also compounds other than pectin are probably responsible for the cholesterol lowering effect of the citrus peels.

Key words Hamsters – Pectin – Lipoproteins – Cholesterol

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Introduction

The cholesterol lowering properties of dietary pectin have been documented in a number of studies. The hypocholesterolemic effect of dietary pectin has been reported in studies with humans [1], rats [2–4], mice [5], guinea pigs [6], hamsters [7, 8], rabbits [9, 10], chickens [9, 11], and pigs [12–14]. There are, however, also studies in rabbits, guinea pigs, hamsters [15–17], monkeys [18], chickens [19], and pigs [20, 21] that did not find such a hypocholesterolemic effect of pectin. Fisher et al. [9] discussed that the hypocholesterolemic properties of dietary pectin are predominantly effective when the

pectin is fed as a part of a cholesterol rich, atherogenic diet. There are, however, also studies that did not find a cholesterol lowering effect of pectin even in the presence of dietary cholesterol [15–17, 20].

Citrus peels are an important source of pectin for industrial use, and the pectins are extracted from the peels. Various types and fractions of pectins can be derived from the citrus peels, and in a previous study we observed that a pectin fraction with a high viscosity was more effective in lowering plasma and liver cholesterol in hamsters than a pectin fraction with a lower viscosity [8]. In the present study, we further studied the effects of pectin on plasma and liver cholesterol levels in the hamster, and we were particularly interested in the cholesterolemic properties of the intact peels in comparison with the pectin derived from these peels. Therefore, we fed hamsters lemon peels, pectins extracted from the peels and the waste stream material that is left over after extracting pectins from the peels. As a control we used cellulose, since cellulose has been shown not to affect plasma cholesterol levels in the hamster [22]. Male hamsters were used because male hamsters are more sensitive to changes in plasma cholesterol levels by dietary intervention than female hamsters [23].

Materials and methods

Animals and diets

Fifty-six male hybrid F_1B Golden Syrian hamsters (6 weeks old) were purchased from BioBreeders Inc., Watertown, MA 02472, USA. On arrival, the hamsters were fed a commercial rodent diet (Hope Farms, 3440 AB Woerden, The Netherlands) for a period of 1 week. Then, body weights, plasma total and HDL cholesterol and triacylglycerol concentrations were measured (see analytical methods). The hamsters were subsequently divided into 4 groups of 14 hamsters with a computer program so that the mean values and the standard deviations of each parameter measured were similar for each group. The groups were subsequently transferred to semipurified diets containing 3 % (w/w) cellulose, lemon peels, lemon pectin, and the waste stream material of the lemon peels. The waste stream material remains after extraction of the pectin from the peels. The various pectin preparations were prepared by Copenhagen Pectin, DK-4623 Lille Skensved, Denmark. The lemon pectin was extracted from the lemon peels with acidified hot water and the extract was clarified by centrifugation and a number of filtrations. The pectin was precipitated from the solution with isopropyl alcohol. Cellulose (powdered cellulose, Arbocel Type B 800) was obtained from J. Rettenmaier & Söhne, Fühlstoff-Fabriken, D-7091 Holzmühle über Eilwangen/Jagst, Germany. The composition of the semipurified diets is given in Table 1 and is similar to that used in a previous study [8]. The hamsters had free access to food and tap water and the diets were fed for a period of 8 weeks. The hamsters were housed in groups of 3 or 4 animals in polycarbonate cages with a bedding of wood shavings. The animal room was temperature controlled (20 °C) and had a 12 hour light-dark cycle (light on from 6–18 hours). The experimental protocol was approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine of Utrecht University in The Netherlands.

Table 1 Composition of the semipurified diets[1]

Ingredient	g/100 g
Casein	20.00
Corn oil	1.00
Palm oil	19.00
Corn starch	25.41
Dextrose	25.41
Cellulose, pectin, peels, or waste stream material[2]	3.00
$CaCO_3$	1.24
$NaH_2PO_4 \cdot 2H_2O$	1.50
$MgCO_3$	0.14
KCl	0.10
$KHCO_3$	0.77
Inositol	0.13
Mineral premix[3]	1.00
Vitamin premix[4]	1.20
Cholesterol	0.10

[1] The air-dried diets contained 1974 kJ metabolizable energy/100 g. The following values were used for the calculation of the energy density of the diet: protein and carbohydrates, 16.74 kJ per gram; fat, 37.66 kJ per gram. The diets contained 100 mg cholesterol per 1,974 kJ. The energy distribution of the diets was: fat, 38 %; protein, 17 %; carbohydrate, 45 %. The calculated P/S ratio of the diets was 0.26.

[2] The fibers were added in hydrated form. Thirty grams of fiber was mixed into 1 liter water and added to 970 grams of the diet ingredients not including the fibers.

[3] Composition in mg/kg of food: $FeSO_4 \cdot 7H_2O$, 174; (Fe, 35), MnO_2, 79, (Mn, 50) $ZnSO_4 \cdot H_2O$, 33 (Zn, 12); $NiSO_4 \cdot 6H_2O$, 13 (Ni, 3); NaF, 2 (F, 1); KI, 0.2 (I, 0.15); $CuSO_4 \cdot 5H_2O$, 15.7 (Cu, 4); $Na_2SeO_3 \cdot 5H_2O$, 0.3 (Se, 0.10); $CrCl_3 \cdot 6H_2O$, 1.5 (Cr, 0.30); $SnCl_2 \cdot 2H_2O$, 1.9 (Sn, 1); NH_4VO_3, 0.2 (V, 0.1); corn meal (carrier material), 9,679.2.

[4] Composition in mg/kg of food: thiamine hydrochloride (vitamin B_1), 4; riboflavin, 3; nicotinamide, 20; calcium pantothenate (purity 45 %), 17.8; pyridoxine hydrochloride, 6; cyanocobalamine (purity 0.1 %), 50; choline chloride (purity 50 %), 2000; folic acid, 1; biotin, 2; menadione (vitamin K), 0.05; all-*rac*-α-tocopheryl acetate (purity 50 %), 60; retinyl acetate and palmitate (500 IU per mg), 8; cholecalciferol (500 IU per mg), 2; corn meal (carrier material), 9,826.15

The semipurified diets were first prepared without the fiber source. Thirty grams of cellulose, lemon peels, or the waste stream material of the lemon peels were added to 1 liter of cold water and mixed in a KitchenAid kitchen machine (Model K5SS/KPM5, KitchenAid Europe Inc., Strombeek-Bever [Brussels], 1853 Belgium). The lemon pectin was added to hot water. The lemon pectin could be completely dissolved and thoroughly mixing the cellulose, lemon peels and waste stream with the cold water resulted in a suspension. Then, 970 g of powdered, air-dried semipurified diet without the fiber source was added and thoroughly mixed in the KitchenAid kitchen machine. A dough-like diet was ob-

tained which was facilitated by the $KHCO_3$ in the diets and which prevented the hamsters from spilling the diets in the cages. The diets were fed to the hamsters every two or three days in feeding bins with an inside-curved edge at the top. This way, the hamsters spilled essentially no food. Food was prepared for the whole experimental period and stored in a freezer at –30 °C. The food intake of 3 or 4 hamsters (we had two cages with 3 hamsters and two cages with four hamsters per dietary group) per 2 or 3 days was measured, and the average food intake per hamster per day for each cage was calculated. The food intakes were averaged for the entire 8-week period.

Analytical methods

Blood was collected every other week. Food was removed at 16.00 hours and any food left in the cheek pouches was also removed. Blood samples were taken the following morning between 10.00 and 12.00 hours into heparinized tubes from the retro-orbital sinus under light anesthesia with ether. At the end of the study, the hamsters were bled from the left ventricle with a 5 ml syringe and an 18 gauge needle under light anesthesia with ether, and the livers were removed.

Plasma cholesterol [24] and triacylglycerol [25] concentrations in individual plasma samples were measured enzymatically on an autoanalyzer (COBAS-BIO, Roche, CH–4002 Basel, Switzerland) with the cholesterol kit (CHOD-PAP method, catalogue No 1489232) and triacylglycerol kit (catalogue No 450032) supplied by Boehringer Mannheim, D–68 305 Mannheim, Germany. Triacylglycerols were measured with corrrection for free glycerol. Plasma very-low-density and low-density lipoproteins (VLDL+LDL) in individual plasma samples were precipitated with phosphotungstic acid/$MgCl_2$ (Sigma Diagnostics, St Louis, MO 63178, USA, catalogue No 352–4) according to Weingand and Daggy [26], and the supernatant (high-density lipoproteins, HDL) was assayed for cholesterol. The concentrations of cholesterol in the VLDL+LDL was calculated as the difference between whole plasma and HDL. At the end of the experiment (8 weeks on the diets), the plasma samples were pooled per group, and the various lipoprotein fractions (LDL, HDL, VLDL) were isolated by density gradient ultracentrifugation [27].

Livers were homogenized with 15 ml distilled water in a turrax blender, and 50 µl of the homogenate were added to 500 µl of ethanol containing 6 % (v/v) of a KOH solution (50 g/100 ml). The mixture was incubated at 37 °C for 55 minutes, and 1 ml of petroleum ether/diethylether (1:1, v/v) was added. The mixture was thoroughly vortexed and centrifuged. Supernatant (700 µl) was removed and evaporated to dryness under nitrogen. The residue was solubilized in ethanol in a sonication bath and the cholesterol was measured enzymatically.

Fecal bile acids and neutral sterols

Fecal sterols in pooled samples (one pool of feces per dietary group) were measured after the hamsters had been fed the diets for 6.5 weeks. Feces were collected over a period of three days. During the period of feces collection, the hamsters were housed on wire mesh bottoms and filter paper was placed at the bottom of the cages underneath the wire mesh bottoms to absorb the urine. This way, the dry feces could be easily and quantitatively collected. Total phytosterols in the diets were also determined by gas chromatography. Lyophilized feces or diet (150 mg) were added to a tube containing 700 µl methanol and 220 µL of a NaOH solution (5 mol/L). 5α-cholestane was used as an internal standard. The mixture was vigorously vortexed and incubated for 2 hours in a shaking waterbath of 80 °C. NaCl was added after cooling to prevent gel formation, and the neutral sterols were extracted with 3 times 3 mL of petroleum ether (fraction with boiling point range of 60–80 °C). After extraction of the neutral sterols, the methanol mixture with the remaining petroleum ether was evaporated to dryness under nitrogen at 80 °C. Then 670 µl of HCl (3 mol/L) was added, and subsequently the bile acids were extracted with 3 times 3 ml of petroleum ether. The bile acid extracts were evaporated to dryness, and the collected bile acids were methylated by adding 500 µl methanol, 500 µl 2,2 dimethoxy propane, and 50 µl concentrated HCl. The mixture was mixed, left overnight for 16 hours, and evaporated to dryness under nitrogen at 80 °C. The neutral sterol extracts were also evaporated to dryness and both the bile acids and the neutral sterols were silylated as described by Setchell et al. [28]. The sterols were analyzed on a Hewlett Packard (HP 5890, Palo Alto, CA, USA) gas chromatograph equipped with a flame ionization detector and a capillary column (DB 1701, 30 m, 0.25 inner diameter, 0.15 µm film thickness). The column was operated at the following temperatures: 50 °C, increase of 6 °C/min to 250 °C, increase of 2 °C/min to 280 °C, then 40 min at 280 °C. Injection temperature was set at 225 °C and detector temperature at 325 °C. Helium gas was used as the carrier at a pressure of 85 kPa. A 1 µl sample dissolved in hexane was injected using a split ratio of 1:100.

Statistical analysis

Plasma lipid concentrations were measured at different time points. Therefore, we used a Two Way (diets and weeks as factors) Repeated Measures Analysis of Variance (ANOVA) on one variable (lipid concentrations) to statistically analyze the differences in lipid concentrations at 2, 4, 6, and 8 weeks after the beginning of the experiment (week 0). The other results were also measured with a One Way (diet as factor) ANOVA on one variable

(measured parameter). A pairwise multi-comparison procedure (t-test with the Bonferroni adaptation) was used to determine the groups or time points that were significantly different when the ANOVA test indicated a significant effect. Correlations between parameters were statistically analyzed with the Pearson product moment correlation test. Statistical analyses were done with the SigmaStat® statistical software package (Version 1.0, Jandel Corporation, San Rafael, CA 94912, USA).

Results

The diets were fed in the form of a dough and were well accepted. The hamsters did not spill any food. Fresh food was provided every 2 or 3 days. The food intake was slightly higher in the hamsters fed cellulose compared with the other groups (Table 2). Body weights were the same in all the groups at the end of the 8 week period.

Feeding cholesterol enriched semipurified diets resulted in an increase of plasma cholesterol levels in all the dietary groups after 2 and 4 weeks on the diets (Fig. 1). Cholesterol concentrations in the cellulose fed hamsters continued to increase after 4 weeks on the diet, whereas cholesterol levels in the other groups had reached a plateau. As a consequence, the hamsters fed the peels, pectin, or waste stream had lower plasma cholesterol levels than the hamsters fed cellulose after 8 weeks on the diets, although this effect was not statistically significant for the group fed the waste stream diet. Plasma triacylglycerol concentrations had also increased in all the dietary groups after feeding the cholesterol-enriched semipurified diets but there were no significant differences between the dietary groups.

Differences in total plasma cholesterol concentrations between the cellulose-fed hamsters and the other dietary groups were reflected in differences in VLDL+LDL cholesterol (Fig. 1). HDL cholesterol concentrations were comparable in all the dietary groups throughout the 8 week period. Separation of VLDL and LDL by ultracentrifugation indicated that these differences in VLDL+LDL cholesterol concentrations between dietary groups were predominantly associated with differences in VLDL (Fig. 2).

Liver cholesterol concentrations and the amounts of total liver cholesterol were significantly lower in the hamsters fed the lemon peels, the lemon pectin, and the waste stream material than in the hamsters fed the cellulose (Table 2). We also calculated the total amount of cholesterol in the plasma assuming that the plasma volume was 4 % of the body weight. There was a significant positive correlation between the total amount of cholesterol in the plasma and that in the liver when all the dietary groups were studied together (Fig. 3).

The excretion of fecal sterol was measured after 6.5 weeks on the diets. The sterols were determined in one pool of feces collected over a period of 3 days. The majority of the fecal sterols consisted of neutral sterols (Table 2). Feeding hamsters the lemon peels, lemon pectin, or the waste stream material of the lemon pectin instead of the cellulose tended to increase the fecal excretion of both the bile acids and the neutral sterols. The percentage increase in bile acid excretion, however, was

Table 2 Various parameters in hamsters fed semipurified diets containing cellulose, peels, pectin, or the waste stream material of the peels for a period of 8 weeks[1]

	Cellulose	Peels	Pectin	Waste
Initial body weight (g)	89±8	89±7	89±5	89±5
Final body weight (g) (8 weeks)	156±11	156±9	153±12	156±7.26
Food intake (g/day/hamster)[2]	15.2±1.0	14.7±1.1	14.6±1.1	14.3±1.1
Liver weight (g)	5.95±0.64	5.61±0.63	5.29±0.58	5.61±0.47
Liver cholesterol (μmol/g liver)	7.19±2.32[a]	3.57±1.01[b]	4.86±1.42[b]	4.96±1.89[b]
Total liver cholesterol (μmol)	43.41±15.81[a]	19.98±6.34[b]	25.48±6.87[b]	27.95±11.24[b]
Total plasma cholesterol[3] (μmol)	42.10±10.29[a]	35.11±5.81[a, b]	31.89±3.71[b]	34.40±5.37[b]
Cholesterol intake[4] (μmol/day/hamster)	19.65	19.00	18.88	18.49
Total fecal sterols[5] (μmol/day/hamster)	26.99	29.64	30.82	33.14
Total neutral sterols (μmol/day/hamster)	25.27	27.18	28.82	30.54
Total bile acids (μmol/day/hamster)	1.72	2.46	2.00	2.60
Difference between sterol intake and excretion (μmol/day/hamster)	7.34	10.64	11.94	14.65
Total phytosterols in food (μmol/g air-dried food)	0.19	0.32	n. d.	0.35

[1] Values are means ± SD, n = 14. Values in a row not sharing a superscript are significantly different ($P < 0.05$). n. d. not determined. Fecal sterols (three day period) in a pool sample were measured after 6.5 weeks on the diets.
[2] Food intake corresponds to 2 times the intake of air-dried diet.
[3] Calculations are based on the assumption that the plasma volume of the hamster is 4 % of its body weight.
[4] The diets contained 0.1 % cholesterol or 1 mg per g air-dried food (2.59 μmol/g air-dried diet).
[5] Fecal sterols were measured in pooled samples (one pool of feces per dietary group).

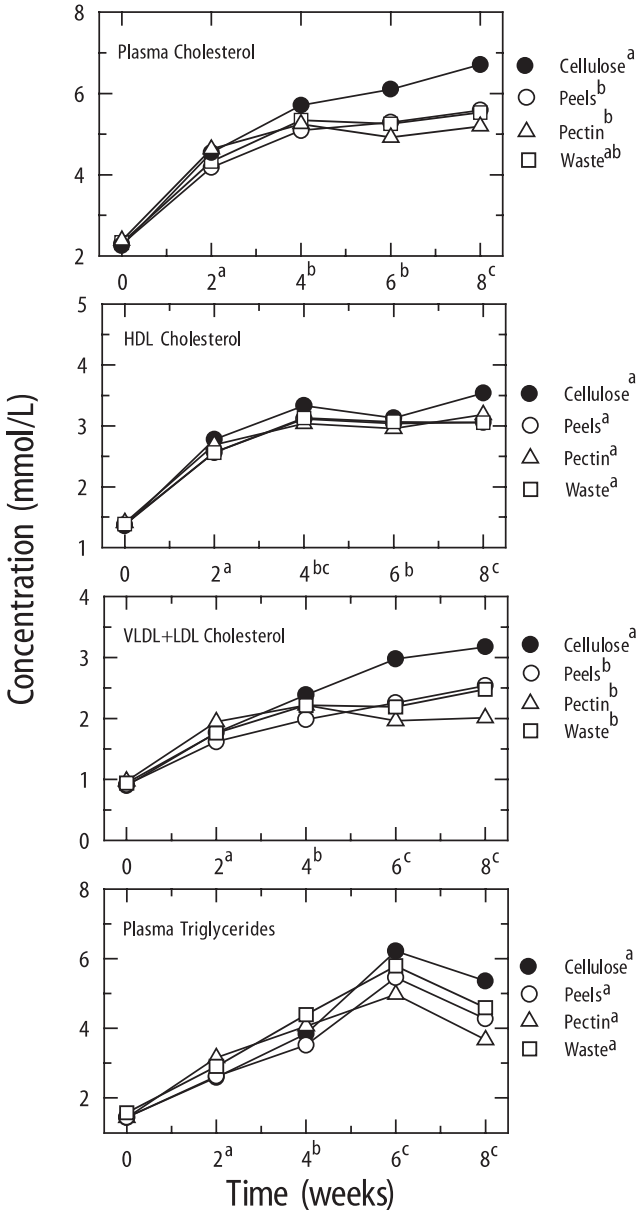

Fig. 1 Plasma concentrations of total cholesterol, high density lipoprotein (HDL) cholesterol, very low density lipoprotein + low density lipoprotein (VLDL + LDL) cholesterol, and triacylglycerol in hamsters fed semipurified diets containing either cellulose, lemon peels, lemon pectin, or the waste stream of the lemon pectin. Values are means of 14 hamsters per group. Dietary groups and time points that do not share a superscript are significantly different ($P < 0.05$).

higher than the percentage increase in neutral sterols. We also calculated the dietary intake of cholesterol and this amount was lower than the fecal excretion of total sterols (Table 2). This difference between the cholesterol intake and the fecal excretion of total sterols may reflect cholesterol from endogenous origin in the hamster if one assumes that a steady state has been reached.

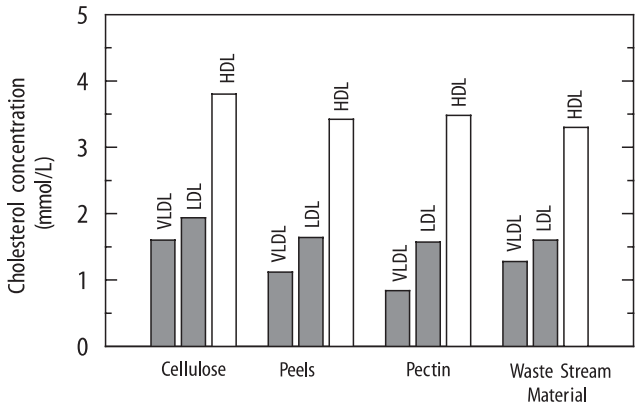

Fig. 2 Cholesterol concentrations in very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL) of hamsters fed semipurified diets containing cellulose, lemon peels, lemon pectin, or the waste stream of the lemon pectin. The results are from pooled samples (14 hamsters per group) taken after the hamsters had been fed the diets for 8 weeks.

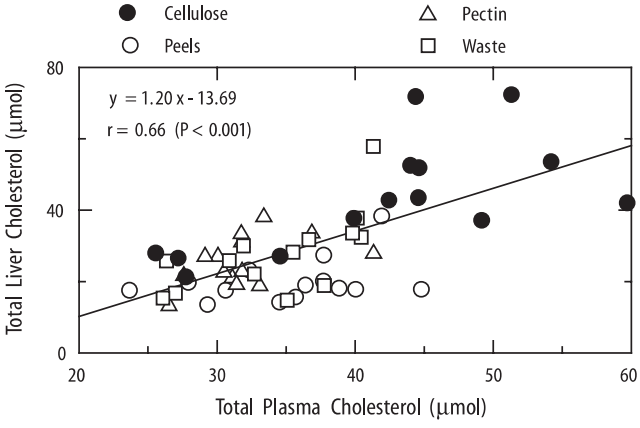

Fig. 3 Correlation between total plasma cholesterol and total liver cholesterol of hamsters fed semipurified diets containing cellulose, lemon peels, lemon pectin, or the waste stream of the lemon pectin.

Discussion

There are only a few studies that have examined the effects of dietary pectins on plasma and liver cholesterol concentrations in hamsters. Sable-Amplis et al. [7] reported that adding 5% apple pectin to the diet of hamsters resulted in a significant reduction of plasma and liver cholesterol. Trautwein et al. [15, 16], on the other hand, found that dietary pectin (8%) did not affect plasma cholesterol in hamsters. We found in previous studies with hamsters that pectin with a high viscosity effectively lowered both liver and plasma cholesterol concentrations [8]. In the present study we similarly observed that feeding pectin extracted from lemon peels resulted in a significant decrease of both plasma and liver cholesterol in hamsters.

Moreover, we found that the lemon peels lowered

plasma and liver cholesterol as effectively as the lemon pectin extracted from the lemon peels. Furthermore, the waste stream material of the lemon peels, i. e., the peels after extraction of the pectin, also lowered considerably plasma and liver cholesterol levels. Both the peels and the waste stream contain pectins, but the proportion of pectin is much lower than that in the pectin preparation. The peels contained 18% galacturonic acid and the waste stream material 15% galacturonic acid which was considerably lower than that of the lemon pectin (79% galacturonic acid). Thus, other factors than pectin might play a role in the cholesterol lowering effect of the peels and the waste stream material. It may be that native pectin as present in the peels is more effective in lowering plasma cholesterol than pectin that has been treated with extraction fluids. Another possibility might be the presence of phytosterols in the peels and the waste stream material. The diet containing lemon peels or the waste stream material contained more phytosterols than the cellulose diet (Table 1), and there is evidence that phytosterols in the diet can lower plasma cholesterol concentrations. Miettinen et al. [29] fed to humans 2,600 mg sitostanol and Pelletier et al. [30] fed 740 mg phytosterols per day. Both studies reported a 10% reduction in plasma cholesterol levels. This amount of phytosterols in these two human studies was equivalent to a consumption of 0.65 and 0.185 mmol phytosterols per MJ of energy, respectively. We calculated that the amount of phytosterols in the lemon peel diet was 0.016 mmol per MJ. These amounts are considerably less than the amounts fed to humans by Miettinen et al. [29] and Pelletier et al. [30]. Thus, it seems unlikely that the phytosterol content of the peels might have been responsible for the cholesterol lowering effect of the lemon peels.

As a consequence, the peels and the waste stream material of the peels might contain other components that lower plasma cholesterol. Citrus peels also contain bioflavonoids such as naringin and hesperidin [31], the glycosylated forms of naringenin and hesperetin, respectively. Ethanol extracts from tangerine peels which contain these two compounds have been reported to lower the activity of hepatic 3-hydroxy–3-methyl-glutaryl-CoA (HMG-CoA) reductase, a rate limiting enzyme in the cholesterol synthesis, and to lower plasma and hepatic cholesterol levels in rats [31]. Similarly, orange juice and grapefruit juice which also contain hesperidin and naringin, respectively, have been shown to lower plasma cholesterol concentrations in rabbits [32]. Thus, the presence of these bioflavonoids could be at least partly responsible for the cholesterol-lowering activity of the peels. Naringin and hesperidin can be extracted from citrus peels with ethanol [31] and the lemon pectin in our study was extracted from the peels with acidified hot water. Thus, most of these two flavonoids were probably not extracted and might have contributed to the cholesterol-lowering effect of the waste stream material.

Furthermore, there are indications that the cholesterol-lowering effect of pectin may be mediated by short chain fatty acids, such as acetate, propionate, and butyrate, which are formed by fermentation of pectin in the intestine. These short chain fatty acids are effectively absorbed and may inhibit cholesterol synthesis [33]. It is possible that the peels and the waste stream material contain carbohydrates or fibers other than pectin that also can be fermented into these short chain fatty acids and affect cholesterol metabolism.

The cholesterol lowering effect of the peels, pectin, and waste stream material was associated with a higher excretion of bile acids and neutral sterol. Trautwein et al. [15, 16] reported no significant effect of pectin on the excretion of fecal bile acids and neutral sterols in hamsters, but in their studies they also did not find an effect of pectin on plasma cholesterol concentrations. We also found in previous studies with hamsters that pectin enhanced the excretion of both bile acids and neutral sterols [8]. Similarly, other studies in humans [34–39], rats [40–43] and mice [5] have shown that dietary pectin increased the excretion of bile acids. In addition, studies in humans [36, 38, 39], rats [41, 42, 44, 45] and mice [5] have indicated that pectin also increased the excretion of neutral sterols in the feces.

If one assumes that cholesterol metabolism in the hamsters had reached a steady state, then the difference between the sterol intake and excretion (Table 2) represents the amount of cholesterol that is endogenously synthesized. Dietschy et al. [46] reported that the synthesis of cholesterol in the hamster is about 25 mg cholesterol per day per kg body weight which is equivalent to 9.70 μmol/day/hamster in a hamster of 150 g. This amount is in the range we found in our studies (7.34–14.65 μmol/day/hamster). The difference between the intake of cholesterol and the fecal excretion of total sterols in the cellulose fed hamsters was lower than that in the other dietary groups, which suggests that the peels, pectin, and waste stream material may have enhanced the endogenous cholesterol synthesis. Studies in rats [40, 45, 47, 48] and guinea pigs [49] have indeed shown that dietary pectin increased the activity of HMG-CoA reductase activity, a rate limiting enzyme in the cholesterol synthesis.

It is, however, not clear whether cholesterol metabolism has already reached a steady state in the hamsters. The increasing levels of plasma cholesterol in the cellulose fed hamsters might indicate that at least in this group the body is still accumulating cholesterol. When there is no steady state and cholesterol is still being stored in the body, then the total cholesterol synthesis will be equal to the difference between the intake and excretion of sterols plus the amount that is being stored in the body. Since the cellulose fed hamsters will accumu-

late more cholesterol in the body than the other groups, the differences in cholesterol synthesis between the cellulose group and the other groups will then become smaller than the differences in cholesterol synthesis estimated on the basis of a steady-state situation (Table 2).

In conclusion, the results of this study suggest that lemon peels and the waste stream of the lemon peels are as effective in lowering plasma and liver cholesterol in hamsters as the pectin extracted from the peels and that compounds other than pectin are probably responsible for the cholesterol lowering effect of the citrus peels.

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