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ORIGINAL ARTICLE

Cholesterol-lowering properties of different pectin types in mildly hyper-cholesterolemic men and women

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Background/Objectives: Viscous fibers typically reduce total cholesterol (TC) by 3–7% in humans. The cholesterol-lowering properties of the viscous fiber pectin may depend on its physico-chemical properties (viscosity, molecular weight (MW) and degree of esterification (DE)), but these are not typically described in publications, nor required by European Food Safety Authority (EFSA) with respect to its generic pectin cholesterol-lowering claim.
Subjects/Methods: Here, different sources and types of well-characterized pectin were evaluated in humans. Cross-over studies were completed in mildly hyper-cholesterolemic persons receiving either 15 g/day pectin or cellulose with food for 4 weeks.
Results: Relative low-density lipoprotein (LDL) cholesterol (LDL-C) lowering was as follows: citrus pectin DE-70 = apple pectin DE-70 (7–10% reduction versus control) > apple pectin DE-35 = citrus pectin DE-35 > OPF (orange pulp fiber) DE-70 and low-MW pectin DE-70 > citrus DE-0. In a subsequent 3-week trial with 6 g/day pectin, citrus DE-70 and high MW pectin DE-70 reduced LDL-C 6–7% versus control (without changes in TC). In both studies, high DE and high MW were important for cholesterol lowering. Source may also be important as citrus and apple DE-70 pectin were more effective than OPF DE-70 pectin. Pectin did not affect inflammatory markers high-sensitivity C-reactive protein (hsCRP) nor plasma homocysteine.
Conclusions: Pectin source and type (DE and MW) affect cholesterol lowering. The EFSA pectin cholesterol-lowering claim should require a minimum level of characterization, including DE and MW.
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Introduction

Pectin: what is it?

Pectin is a complex polysaccharide present in plant cell walls, extracted commercially from pulp waste during fruit juice pressing (for example, from citrus peel and apple pomace). After extraction and processing, different molecular compositions and functional characteristics are obtained, impacting gelation, thickening, food stabilization and mouth feel. Pectin is a gelling agent in jams, marmalades and reduced sugar versions. Pectin is used in fruit-containing preparations (for example, yogurts and desserts) to create thickened textures and a homogenous distribution of fruit pieces. Pectin is thus a texturizing and gelling agent. Gastrointestinal gelling affects transit rate, nutrient absorption rate, and cholesterol absorption and secretion. A 3–7% reduction in total cholesterol (TC) is achievable with various gelling fibers (see below).

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Pectin types and processing

Methyl esters in pectin chains are expressed as degree of esterification (DE). High-methoxyl pectin has >50% DE and form viscous gels in the stomach at pH 2.2–3.5 (Ralet *et al.*, 1994; Sila *et al.*, 2009). Low-methoxyl pectin has <50% DE. High-methoxyl and low-methoxyl pectin represent the two major types. High-methoxyl pectin form gels with high sugar content (>60%) at low pH. Low-methoxyl pectin

forms gels with divalent cations (notably Ca^{2+}) at pH 2.5–6 (sugar addition not required) (Thibault and Ralet, 2008). Further processing (for example, saponification, amidation) is used to obtain desired viscosities/gelation (Thibault and Ralet, 2008). Overall, various processing factors, including DE, molecular weight (MW; high: HMW and low: LMW) and pectin starting source (for example, citrus versus apple) (Baker, 1997; Duvetter *et al*., 2009), may affect cholesterol-lowering efficacy in pectin. Pectin types used in clinical trials have however been rarely well-described.

Pectin lowers cholesterol in animal models

Pectin lowers blood and liver cholesterol in various animals, including guinea pigs (Gorinstein *et al*., 2005), rats (Krzysik *et al*., 2011), hamsters (Terpstra *et al*., 2002), chickens (Craig *et al*., 2006) and rabbits (Ismail *et al*., 1999). Based on results from several animal studies (Yamaguchi *et al*., 1995; Trautwein *et al*., 1998; Dongowski and Lorenz, 2004), it has been stated that the molecular composition of pectin affected cholesterol reductions in blood and liver. To determine whether the molecular composition of pectins to be tested in a subsequent human clinical trial affected cholesterol-lowering, nine different pectin types were evaluated in 486 male broilers for 35 days using a parallel study design (Craig *et al*., 2006). Ranking for serum cholesterol-lowering efficacy on days 21 and 35 was as follows: citrus pectin DE-70 = apple pectin DE-7 > apple pectin DE-35 > citrus pectin DE-0 > low-MW pectin > citrus pectin DE-35 > cellulose. Clearly, the molecular composition of these pectins affected cholesterol-lowering efficacy.

Pectin lowers cholesterol in humans

Human studies of pectin cholesterol lowering are scarce relative to the animal literature (Cerda, 1988; Cerda *et al*., 1988). In general, each gram of pectin lowers LDL cholesterol (LDL-C) by 0.055 mmol/l (Brown *et al*., 1999). However, in most human studies the effects of pectin were variable and high intakes of more than 10 g/day were used, an impractical level for food incorporation. Pectin was also not well-characterized in the above studies.

Cholesterol-lowering claim

In 2010, the Panel on Dietetic Products, Nutrition and Allergies of the European Food Safety Authority (EFSA) (EFSA, 2010) published its Scientific Opinion on substantiation of health claims related to pectin, including cholesterol lowering and effects on glycemia. For cholesterol lowering (maintenance of normal blood cholesterol concentrations), a cause-and-effect relationship was established with 6 g pectin in $\geqslant 1$ servings. EFSA considered a meta-analysis on pectin by Brown *et al*. (1999), including seven studies, and a review by Reiser (1987), including 18 studies.

In the meta-analysis (Brown *et al*., 1999), there was a statistically significant dose response lowering effect of pectin on TC and LDL-C at 2.2–9 g/day. One gram pectin per day was estimated to lower LDL-C 0.05 mmol/l. LDL-C was considered in four of seven studies.

In the review of 18 studies (Reiser, 1987), the authors evaluated the effects of 2–40 g pectin per day. In 14/18 studies, pectin significantly lowered TC. LDL-C was generally not evaluated. In a dose–response study of 16 subjects, 6–10 g pectin per day significantly reduced TC 4–6%; effects with 2–4 g/day were not statistically significant (Palmer and Dixon, 1966). The mechanism by which pectin lowered cholesterol is likely similar to other water-soluble fibers. By increasing gut viscosity, it reduces the re-absorption of bile acids, increasing synthesis of bile acids from cholesterol, and thereby reduces circulating blood cholesterol. The positive opinion from EFSA does not consider pectin characterization, a likely factor contributing to observed variability in TC and LDL-C lowering. DE and MW are examples of physico-chemical factors in pectin impacting cholesterol lowering. Also, EFSA does not consider difficulties ingesting > 6 g/day pectin in foods owing to taste concerns and potential gastrointestinal disturbance. Finally, EFSA relied on earlier literature, focused on TC but not LDL-C, the latter a better indicator of cardiovascular disease.

In view of EFSA's opinion, it is timely and important to understand how pectin's structure affects cholesterol lowering. Here, pectin from different native sources was subsequently modified by processing, resulting in pectins with different DE and MW, for evaluation in two human clinical trials, under controlled conditions.

Materials and methods

Inclusion and exclusion criteria (PEC1 and PEC2)

The primary inclusion criteria were as follows: body weight index $< 32\,kg/m^2$, diastolic blood pressure < 95 mm Hg or systolic < 160 mm Hg, mean TC > 5.0 and < 8.0 mmol/l, mean triacylglycerol (TAG) < 4.0 mmol/l, and no presence of glucosuria. The exclusion criteria were as follows: body weight index $> 32\,kg/m^2$, diastolic blood pressure > 95 mm Hg or systolic > 160 mm Hg, mean serum TC < 5.0 mmol/l and TAG > 4.0 mmol/l, and indication for treatment with cholesterol-lowering drugs according to the Dutch cholesterol consensus (Centraal Begeleidingsorgaan voor de Intercollegiale Toetsing, 1998).

Screening visits (PEC1 and PEC2)

Prior to studies, subjects completed one or two health screenings for determination of body weight, height, blood pressure (diastolic and systolic), serum TC, LDL-C and high-density lipoprotein (HDL) cholesterol (HDL-C), TAG, glucosuria, use of medication, current diseases and history of coronary disease. If diastolic blood pressure was 85 or systolic above 150 mm Hg, blood pressure was measured at both screening visits (Table 3).

Informed consent, withdrawal, diaries, ethical approval (PEC1 and PEC2)
Before experimentation, volunteers were given oral and written information about study aims, and signed an informed consent. Subjects were permitted to approach an independent physician for further information and questions, and to withdraw without explanation. Subjects reported signs of illness, medications, menstrual phase and protocol deviations in diaries. Subjects were urged not to change levels of physical exercise, smoking, or use alcohol or oral contraceptives during study. The studies were approved by the ethical medical committee of the Maastricht University Medical Center.

Composition and intake of experimental products (PEC1)
The commercial and experimental pectins used in PEC1 and PEC2 were characterized by standard analytical methods to determine MW, and DE, according to JECFA (2009). In PEC1, subjects received pectin-containing foods to provide 15 g/day of cellulose or different types of pectin. Pectin was incorporated into cereal bars, fruit preparations and capsules. On the basis of taste, appearance, smell and quantity consumed the various types of pectin or cellulose in the bars and fruit preparations were judged to be acceptable on a scale of 1 (bad) to 5 (good), with a range of 2.6–4.0, with slightly lower scores for citrus DE-0 and citrus DE-35 in the fruit preparations (1.5–2.0). The composition of the 25-g cereal bar (one serving, 85 kcal) was as follows: 1 g total fat (containing 0.125 g saturated fat), 20.5 g total carbohydrate (containing 5.75 g sugar and 2.5 g dietary fiber all of which was pectin), 0.75 g protein and 147.5 mg sodium. The composition of the fruit preparation per 200 ml serving (240 kcal) was as follows: 0.6 g sodium citrate dihydrate, 50 g fructose, 100 g frozen or fresh cherries, 5 g pectin as a hydrocolloid, and water to a final volume of 200 ml after cooking. Capsules were provided in blister packs. Each 0.6-g capsule contained 0.5 g pectin powder, 18.0 mg protein, 4.8 mg sodium, 0.7 mg potassium, 3.0 mg calcium, 0.4 mg magnesium and 69.1 mg water. Subjects consumed the daily 15 g of pectin by a self-chosen combination of products. For example, 4 bars and 1 fruit preparation; and 2 bars, 1 fruit preparation and 10 capsules. Bars were consumed with drinks, either at breakfast, or later in the day as a snack between meals with coffee or tea. The fruit preparation was mixed with milk, fresh cheese or yogurt. Capsules were consumed together with a dairy product. Subjects were advised to consume experimental products instead of habitual diets, so total daily energy intake remained stable.

Composition and intake of experimental products (PEC2)
In PEC1, subjects found fruit gel preparations to be too voluminous. Thus, in PEC2, pectin (citrus DE-70 or citrus HMW DE-70) or cellulose (control) was provided in capsules consumed with 200 ml of beverage, divided over breakfast, lunch and dinner. The capsules were identical to those of PEC1 except pectin per capsule was reduced to 0.325 g. Capsules were provided in a blister pack containing nine capsules providing 3 g pectin. Subjects received two blister packs of capsules providing 6 g pectin or cellulose per day.

Product distribution, diaries, side effects (PEC1 and PEC2)
Subjects received a supply of test products at an instruction visit 1 week prior to study initiation. Products were color-coded, with a sticker to blind the subjects and investigators. Foods not consumed in the previous testing period were returned and counted for compliance. During the experimental phases, subjects recorded in a diary any side effects (headache, stomach complaints, nausea, bloated feeling, flatulence, diarrhea, constipation, itching, eruptions/rashes, fatigue and dizziness), and recorded the time the products were consumed each day for compliance.

Randomization, blinding, treatment allocation (PEC1 and PEC2)
Before study initiation, subjects were randomly allocated to a test group. Random sampling without replacement was used, with subjects stratified for gender, age and body mass index (Snedecor and Cochran, 1980).

Experimental design (PEC1)
PEC1 consisted of a four-period crossover design with three sub-studies consisting of four sequences each (Table 1). There were 30 men and women per sub-study (25–29 finished). Seven different pectin types were compared in total with a cellulose control fiber: three different types of citrus pectin (citrus DE-0, citrus DE-35, citrus DE-70), two different types of apple pectin (apple DE-35 and apple-DE-70), one orange pulp fiber (OPF DE-70) and one LMW pectin (LMW DE-70).

Table 1 Experimental design PEC1

PEC1	*Period-1*	*Period-2*	*Period-3*	*Period-4*
Sub-A1 ($n=7$)	Control	Citrus DE-70	Apple DE-70	Apple DE-35
Sub-A2 ($n=7$)	Apple DE-35	Control	Citrus DE-70	Apple DE-70
Sub-A3 ($n=8$)	Apple DE-70	Apple DE-35	Control	Citrus DE-70
Sub-A4 ($n=8$)	Citrus DE-70	Apple DE-70	Apple DE-35	Control
Sub-B1 ($n=7$)	Control	Citrus DE-0	Citrus DE-35	Citrus DE-70
Sub-B2 ($n=7$)	Citrus DE-70	Control	Citrus DE-0	Citrus DE-35
Sub-B3 ($n=8$)	Citrus DE-35	Citrus DE-70	Control	Citrus DE-0
Sub-B4 ($n=8$)	Citrus DE-0	Citrus DE-35	Citrus DE-70	Control
Sub-C1 ($n=7$)	Control	LMW DE-70	Citrus DE-70	OPF DE-70
Sub-C2 ($n=7$)	OPF DE-70	Control	LMW DE-70	Citrus DE-70
Sub-C1 ($n=8$)	Citrus DE-70	OPF DE-70	Control	LMW DE-70
Sub-C1 ($n=8$)	LMW DE-70	Citrus DE-70	OPF DE-70	Control

PEC1 consisted of a four-period crossover design with three sub-studies consisting of four sequences each. Subjects were randomly assigned to one of four sequences. *n*, number of starting subjects.

Table 2 Experimental design for PEC2

Test groups	*Period-1*	*Period-2*	*Period-3*
Group-1 ($n=10$)	Control fiber	Citrus DE-70	HMW citrus DE-70
Group-2 ($n=10$)	HMW citrus DE-70	Control fiber	Citrus DE-70
Group-3 ($n=10$)	Citrus DE-70	HMW citrus DE-70	Control fiber

Abbreviation: *n*, number of starting subjects.

Each period was 4 weeks, with washout between treatments $\geqslant 1$ week. A cellulose control and citrus DE-70 were included in all sub-studies. In sub-study-A, pectin with the same DE but different source, or same source (apple) but different DE, was compared. In sub-study-B, pectin with same source (citrus) but different DE was compared. In sub-study-C, pectin with the same DE (70) but different source, or with the same DE but different MW, was compared.

Experimental design (PEC2)

A randomized, crossover design was used with three experimental periods of 3 weeks and a washout period of $\geqslant 1$ week in which 30 men and women (27 finished) aged 18–70 years with slightly elevated cholesterol were randomly assigned to one of three sequences to receive cellulose control, citrus DE-70 and citrus HMW DE-70 (same DE and source, but different MW) (Table 2).

Power calculations, statistics, outliers, distribution (PEC1 and PEC2)

Pectin (15 g/day) was estimated to decrease LDL-C by 14% versus control (0.47 mmol/l) (Cerda, 1988). Within-subject variability in LDL-C was estimated at 10% (0.35 mmol/l). It was assumed that the most effective fiber would reduce LDL-C twice the least effective fiber (7% reduction in LDL-C). Assuming a 10% dropout rate, there was 80% power to detect differences in efficacy between three fibers with $n=30$ subjects. For each outcome variable, values outside ± 2.5 s.d. of mean were pre-defined as outliers. Assumptions of normality of residuals and homogeneity of variance were investigated for each outcome variable using Shapiro–Wilkinson (Shapiro and Wilk, 1965) and Levene's tests (Levene, 1960), respectively, prior to analysis of variance (ANOVA). If either hypothesis was rejected ($P\leqslant 0.05$), distribution was then examined using normal quantile–quantile and kernel density plots (Peng, 2004). Outcome variables not following normal distribution assumptions were analyzed by ranking values prior to ANOVA. These variables included the following: PEC1: Sub-study-A: TAG and TC/HDL-C; PEC1: Sub-study-B: TAG and TC/HDL-C; PEC1: Sub-study-C: HDL-C and TAG; and PEC2: TAG, high-sensitivity C-reactive protein (hsCRP) and homocysteine.

In PEC1, for each lipid parameter, descriptive statistics (number of subjects, mean, standard error of the mean, s.d., median, inter-quartile range, minimum and maximum values) were calculated for 0-, -21, -28 days, mean of 21 and 28 days, and differences and percent difference for mean of 21 and 28 days, versus control. In PEC2, for each lipid parameter, descriptive statistics were calculated for 0-, -17, -21 days, mean of 17 and 21 days, and differences and percent difference for mean of 17 and 21 days, versus control. Similar statistical conclusions were reached using final values as compared with the mean of the last two measurements (data not shown). The tables show the statistics for the mean of the last two measurements.

For each outcome variable, repeated-measures ANOVA modeling was used. The initial repeated-measures ANOVA model contained terms for baseline value (day 0), intervention, sequence and period. Models were reduced stepwise until statistically significant terms ($P\leqslant 0.10$), for intervention and baseline values, remained. Pairwise comparisons to control intervention were adjusted by Dunnett's test and *P*-values were considered at $P<0.01$, $P<0.05$ and $P<0.1$ (the latter to identify statistical trends). Appropriate covariance structures were determined by using the Akaike information criterion (Akaike, 1974, 1987). The Akaike information criterion selects the model with the smallest Akaike information criterion. Carry-over determination was made at day 0 using repeated-measures ANOVA. Occurrences of headache, nausea, stomach bloating, flatulence, diarrhea, constipation, stomach complaints, collywobbles, etc. (PEC1, PEC2), were summarized by number and percentage of subjects, and analyzed by repeated-measures categorical modeling.

Blood sampling and analyses (PEC1 and PEC2)

Two blood samples were drawn during screening, 12 samples were drawn during PEC1 and 9 were drawn during PEC2. All samples were collected by venipuncture and to the extent possible, by the same technician and at the same location, at the same time of day. Serum and plasma were obtained by low-speed centrifugation within 1 h after venipuncture, and stored. All samples from one subject were analyzed within one run at study end. For PEC1, blood samples were collected at 0, 7, 21 and 28 days. For PEC2, blood samples were collected at 0, 17 and 21 days. In both studies, blood was analyzed for: LDL-C, TC, HDL-C and TAG. For PEC2, in addition, hsCRP was assessed at 0, 17 and 21 days, and homocysteine at 17 and 21 days. Samples were analyzed for TC (Cobas Mira, CHOD-PAP method; Roche diagnostics Systems, Hoffmann-La Roche Ltd, Basel, Switzerland), HDL-C (precipitation method) and TAG with correction for free glycerol (GPO Trinder; Sigma Aldrich Chemie BV, Zwijndrecht, Netherlands). TC/HDL-C was calculated and LDL-C was estimated (Friedewald *et al*., 1972). hsCRP was measured using the Cobas Mira with a kit (Kamiya Biomedical, Seattle, WA, USA). Homocysteine was reduced (Immuchrom, Heppenheim Hessen, Germany) and albumin bound homocysteine was liberated. After reaction with free sulfate groups and derivatization with SBD-F (ammonium 7-fluorbenzo-2-oxa-1,3-diazole-4-sulfonate), homocysteine

was quantified fluorospectroscopically after high-performance liquid chromatography (with a C18 reversed-phase column and fluorescence detection; excitation: 385 nm and emission: 515 nm) (Hagan, 1993). Body weight was recorded at the end of each dietary period in both studies.

Results

Dropout rates, baseline values, compliance (PEC1 and PEC2)
In PEC1 (9/90) and PEC2 (3/30) the dropout rate of 10% was predicted in power calculations, and was caused by personal circumstances or disease. The baseline values of individuals enrolled in PEC1 and PEC2 were similar, and mean subject characteristics of subgroups, and distribution of men and women, were nearly identical (Table 3). Compliance for ingesting the requested amounts/types of fiber was high in PEC1 and PEC2. In PEC1, pectin intake was set at 15 g/day. Actual intake (g, mean ± s.d.) was as follows 14.0 ± 1.0, 14.7 ± 0.6 and 14.3 ± 1.1 for cellulose control in sub-A, B and C, respectively; 13.8 ± 2.0, 14.2 ± 1.2 and 14.2 ± 1.1 for citrus DE-70 in sub-A, B and C, respectively; 13.9 ± 1.7 for apple DE-70; 14.2 ± 1.1 for citrus DE-0; 13.9 ± 1.4 for citrus DE-35; 14.4 ± 1.3 for apple DE-35; 14.3 ± 1.3 for LMW DE-70; and 14.6 ± 0.5 for OPF DE-70. In PEC2, pectin intake was set at 6 g/day. Actual intake in PEC2 was as follows: 5.8 ± 0.2 g for control, 5.9 ± 0.3 g for citrus DE-70 and 5.9 ± 0.2 g for citrus HMW.

Impact of statistical outliers (PEC1 and PEC2)
For PEC1, subjects 5, 7, 48, 88, 105, 116, 126, 128 and 133 had $\geqslant 1$ outlying value (>2.5 s.d. from mean). For PEC2, subjects 3, 6, 7, 10, 15, 17, 18, 23, 25 and 28 had $\geqslant 1$ outlying value. Separate analyses were generated for each study excluding outlying subjects. Analyses excluding outlying values were similar to results including all values.

Effects on cardiovascular disease risk factors (PEC1)
Relative to control there were significant reductions in TC, LDL-C and TC/HDL-C for apple DE-70 and citrus DE-70, and these two pectin types were generally most effective (Table 4). Apple DE-70 and citrus DE-70 were equally effective ($P<0.1$). Citrus DE-35 and citrus DE-70 had similar effects on TC and LDL-C, whereas in apple pectin, DE-70 was more effective than DE-35. Citrus DE-0 had no effects on measured parameters. Relative to control, OPF DE-70 did not lower

Table 3 Baseline characteristics of test persons in PEC1 (sub-studies A–C) and PEC2

PEC1	*Sub-A*	*Sub-B*	*Sub-C*	*PEC2*
Number (M/F)	29 (12/17)	27 (13/14)	25 (13/12)	27 (15/12)
Age (years)	58 ± 10	58 ± 8	57 ± 7	60 ± 9
BMI (kg/m^2)	26 ± 3	25 ± 3	25 ± 3	26 ± 3
Systolic blood pressure (mm Hg)	136 ± 14	131 ± 12	134 ± 13	130 ± 16
Diastolic blood pressure (mm Hg)	85 ± 7	84 ± 7	84 ± 6	86 ± 11
TC (mmol/l)	6.55 ± 0.88	6.71 ± 1.01	6.63 ± 0.89	6.89 ± 1.31
LDL-C (mmol/l)	4.43 ± 0.83	4.60 ± 0.99	4.71 ± 0.80	—
HDL-C (mmol/l)	1.50 ± 0.48	1.54 ± 0.50	1.35 ± 0.27	1.52 ± 0.47
TAG (mmol/l)	3.21 ± 1.63	2.90 ± 1.09	2.90 ± 0.98	1.80 ± 0.73

The values represent the means ± s.d. —, not measured. In PEC1 (all sub-studies) and PEC2, the randomization scheme resulted in no significant differences in subjects at baseline between each test group, when beginning Period-1. Baseline values were not used as day 0 values for calculating changes in lipid parameters during the actual study.

Table 4 PEC1 sub-studies: Percent changes versus control for each sub-study

PEC1 study	*Sub-A*			*Sub-B*			*Sub-C*		
Group	*A DE-35*	*A DE-70*	*C DE-70*	*C DE-0*	*C DE-35*	*C DE-70*	*OPF DE-70*	*LMW DE-70*	*C DE-70*
TC (mmol/l)	−3.73 (7.07)	−6.54 (6.49)***	−7.16 (7.21)***	−0.23 (7.31)	−4.65 (6.00)***	−3.99 (7.11)***	−0.45 (5.90)	−0.96 (7.29)	−4.90 (7.38)**
LDL-C (mmol/l)	−4.94 (8.55)**	−9.26 (9.71)***	−10.20 (8.76)***	−0.19 (9.66)	−6.03 (8.75)***	−6.47 (10.29)***	−0.82 (7.47)	−3.05 (9.03)*	−7.24 (8.68)**
HDL-C (mmol/l)	0.62 (7.53)	−0.31 (8.03)	0.99 (9.33)	−1.41 (7.68)	−0.31 (10.37)	0.91 (9.81)	−3.99 (7.17)**,[a]	−1.71 (7.43)	−4.43 (9.65)
TAG (mmol/l)	−3.73 (7.07)	1.08 (25.96)	−3.18 (21.13)	4.32 (35.80)	−5.56 (25.12)	4.91 (37.38)	18.60 (33.59)	16.84 (33.76)	14.24 (26.87)
TC/HDL-C	−4.00 (7.90)*,[a]	−5.74 (8.84)**,[a]	−7.47 (9.51)***,[a]	1.78 (10.84)	−2.98 (12.35)**	−3.97 (11.88)**	4.18 (8.76)**	0.62 (7.82)	0.09 (9.54)
LDL-C/HDL-C	−5.15 (9.76)**,[a]	−8.58 (11.14)***,[a]	−10.44 (11.16)***,[a]	1.84 (12.77)	−3.98 (16.20)**	−6.27 (14.66)**	3.73 (9.87)*	−1.45 (10.12)	−2.26 (11.85)

Abbreviations: A, apple; C, citrus; DE, degree of esterification; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein cholesterol; LMW, low molecular weight; OPF, orange pulp fiber; TAG, serum triacylglycerol; TC, total cholesterol.
The values are the means of days 21 and 28. Comparisons between each treatment are not shown in the table but explained in the text. Statistical coding: *<0.1; **<0.05; ***<0.01. No asterisk: $P>0.1$. *P*-values were adjusted by Dunnett's test. The values within parentheses denote s.d.
[a]Values ranked prior to analysis.

TC and LDL-C, and LMW DE-70 did not affect TC and marginally decreased LDL-C 3% ($P<0.1$). OPF DE-70 had negative effects in reducing HDL-C ($P<0.05$) and trended ($P<0.10$) to increase LDL-C/HDL. TAG increased with LMW DE-70, OPF DE-70 and citrus DE-70 in sub-C, but statistical significance was not achieved ($P<0.1$). As compared with citrus DE-70 and apple DE-70, LMW DE-70 and OPF DE-70 were statistically different for effects on the following: TC and LDL-C ($P<0.01$), TAG ($P<0.05$, $P<0.1$, depending on specific comparison), LDL-C/HDL-C ($P<0.01$, $P<0.05$, $P<0.01$), and TC/HDL ($P<0.01$, $P<0.05$). OPF DE-70, but not LMW DE-70, affected HDL-C ($P<0.1$).

As distribution of cholesterol parameters was similar in sub-A–C, citrus DE-70 data were pooled ($n=80$). In pooled data, there were statistically significant ($P<0.01$) percent reductions in TC, LDL-C and TC/HDL-C of $5.41\pm7.27\%$, $8.05\pm9.31\%$ and 4.02 ± 10.70 (mean ± s.d.), respectively, in the Citrus DE-70 condition, relative to control. HDL-C (-0.71 ± 9.80) and TAG ($+4.89\pm29.63$) were not affected ($P>0.1$). Thus, similar effects were observed for citrus DE-70 using pooled and non-pooled data.

Effects on cardiovascular disease risk factors (PEC2)

Citrus DE-70 and citrus HMW DE-70 decreased LDL-C by 7.22% and 8.84% versus control, respectively. Despite similar percentage reductions, and in contrast to PEC1, statistical significance was only achieved for citrus HMW DE-70 ($P<0.01$) (Table 5). TC/HDL-C ($P<0.1$) and LDL-C/HDL-C ($P<0.05$) were however lowered with citrus DE-70. Citrus HMW DE-70 also lowered LDL-C/HDL-C ($P<0.01$). TC, hsCRP and homocysteine were unaffected by treatments, whereas TAG showed a trend ($P<0.1$) to increase with citrus HMW DE-70; high variability was observed.

Post-consumptive side effects (PEC1 and PEC2)

No statistically significant differences were observed in PEC1 and PEC2 for headache, nausea, stomach bloating, diarrhea, constipation, stomach complaint and collywobbles versus control ($P<0.1$). In PEC1, flatulence, a possible index of pectin colonic fermentation, was reported more frequently with citrus DE-70 (48.0–77.8%) than apple DE-35 and apple DE-70 (<33%; <20% for control). In PEC1, treatments did not affect body weight, with no differences in body weight after consumption of each 4-week feeding of different pectin types ($P<0.1$); body weights ranged from 73 to 75 kg. Similarly, there were no treatment differences in body weight in PEC2.

Table 5 PEC2: Percent changes versus control for means of days 17 and 21

Group	*Citrus DE-70*	*Citrus HMW DE-70*
TC (mmol/l)	−5.24 (7.95)	−5.35 (7.78)
LDL-C (mmol/l)	−7.22 (9.50)	−8.84 (10.61)***
HDL-C (mmol/l)	−0.63 (7.15)	−1.44 (6.28)
TAG (mmol/l)	4.82 (26.91)	13.46 (27.27)*,a
TC/HDL-C	−4.18 (10.71)*	−3.75 (8.29)
LDL-C/HDL-C	−6.03 (13.05)**	−7.27 (12.04)**
hsCRP (mg/l)	22.80 (131.42)	58.36 (179.22)
hsCRP (mg/l) excl. values >7	2.41 (39.96)	9.39 (46.86)
Homocysteine (μmol/l)	1.22 (15.20)	0.30 (18.91)

Abbreviation: hsCRP, high-sensitivity C-reactive protein.
See Table 4 for abbreviations and coding.

Discussion

Cholesterol lowering in animals: mechanisms of action

Pectin has been studied more invasively and mechanistically in animal models than in humans. In rats, pectin increased fecal bile acid excretion, and reduced plasma TAG (−54%), plasma TC (36%), hepatic TAG (−25%), hepatic free and esterified TC (−32%), hepatic bile acid synthesis, and cholesterol synthesis (Vergara-Jimenez *et al*., 1999). In male Wistar rats, pectin decreased serum TC and liver TC (−27 and −17%, respectively), and increased fecal bile acid excretion (+168%), hepatic cholesterol 7-α-hydroxylase (+70%) and HMG-CoA reductase activity (+11%) (Garcia-Diez *et al*., 1996). Guinea pigs fed a high-cholesterol diet and 7.5–12.5% pectin, had a dose-dependent reduction in plasma TC, LDL-C and small dense cholesterol-depleted LDL particles relative to 0% pectin control; HDL-C was not affected (Fernandez *et al*., 1994). Pectin reduced cholesterol absorption 40% and 26%, respectively, in response to low- and high-cholesterol diets. Pectin significantly reduced plasma and hepatic cholesterol ($P<0.001$), and upregulated 7-α-hydroxylase activity in guinea pigs given low and high cholesterol (Fernandez, 1995). Pectin in the high-cholesterol diets increased plasma LDL fractional catabolic rates ($P<0.01$), with no effect on LDL apoB flux rates or pool size, suggesting that LDL-C was reduced without affecting the numbers of LDL particles. Overall, pectin effects cholesterol homeostasis in animals by various mechanisms some of which may operate in humans.

Pectin's molecular characteristics affect cholesterol metabolism in animals

Animal studies also consistently show that the molecular characteristics of pectin affect cholesterol metabolism. Greater reductions in blood and liver cholesterol, and greater fecal excretion of sterols, have been observed with high- versus low-viscous pectin (Terpstra *et al*., 2002). Viscous, gelatinizing citrus pectin modifies bile acid enterohepatic circulation, increasing cholesterol excretion into stool (Martinez de Prado *et al*., 1981; Ide and Horii, 1989; Ide *et al*., 1990; Fernandez *et al*., 1994; Terpstra *et al*., 2002). Highly esterified pectin induced greater reductions in plasma TC and TAG in Syrian hamsters than less esterified pectin (Trautwein *et al*., 1998). In conventional and germ-free rats

(Dongowski and Lorenz, 2004) with increasing degree of methylation (34.5–92.6%), more bile acids were transported into the lower intestinal tract and excreted; secondary bile acids decreased. Pectin with LMW, low viscosity and high solubility was ineffective in reducing TC versus those with HMW and viscosity (Yamaguchi *et al*., 1995). In our pre-screening work (Craig *et al*., 2006), broilers fed a 1% cholesterol diet had a significant increase ($P<0.05$) in serum TC on days 21 and 35, relative to those fed diet without cholesterol ($P<0.05$). Ranking for TC-lowering efficacy of pectin types with differing degree of MW and DE, on days 21 and 35 ($P<0.05$), was as follows: citrus pectin DE-70 = apple pectin DE-70 > apple pectin DE-35 > citrus pectin DE-0 > LMW pectin > citrus pectin DE-35 > cellulose. In a subsequent dose–response study, broilers received 1, 2 or 3% dietary pectin, and ranking for TC reduction was similar to that in the first study with 3% pectin (Craig *et al*., 2006). Overall, the degree of methylation, MW and viscosity strongly impact cholesterol-lowering efficacy of pectin in animal models. *In vitro*, high-DE pectin chelates bile acids and increases intestinal lumen viscosity, supporting these animal findings mechanistically (Dongowski, 1997).

Cholesterol lowering in human trials

Cholesterol lowering by pectin has been reviewed previously (Reiser, 1987; Brown *et al*., 1999; Theuwissen and Mensink, 2008) and its mechanisms of action has been discussed (Gunness and Gidley, 2010). In the review by Reiser (1987), he reported substantial variability in numbers of subjects ($n = 6$–30) and pectin intake (2–40 g/day). During this time period, TC lowering was the main biomarker focus, and TC was lowered in 14/18 evaluated studies. Characterization of pectin types used, apart from source, was not described.

In a more recent meta-analysis of randomized controlled trials (Brown *et al*., 1999), the TC-lowering properties of dietary fibers, including pectin, was considered. In the studies evaluated, initial serum TC and LDL-C were 5.62 ± 0.7 and 4.01 ± 0.59 mmol/l, respectively. Pectin dose was 2.2–9 g/day (mean = 4.7) over 28–42 days. Pectin significantly lowered TC and LDL-C at 2.2–9 g/day. Results between studies were variable, and may reflect the different pectin types used, which was not described in the papers.

The main motivators for conducting our controlled clinical studies on pectin cholesterol lowering were, thus, as follows: the lack of pectin characterization used in clinical trials, despite evidence from animal trials that this is important; the highly variable doses and high pectin doses (unrealistic from a sensory and food formulation perspective), yielding variable lipid lowering; the reliance on too few subjects in clinical trials (statistically underpowered); and the reliance on TC as a primary lipid parameter, rather than LDL-C and other markers. Our studies evaluating different types of pectin (varying by source, MW and DE) took on increasing importance after the recent EFSA claim for cholesterol lowering of pectin, without consideration of source, MW and DE.

In our first study (PEC1), the relative LDL-C-lowering efficacy at a daily intake of 15 g was as follows: citrus DE-70 = apple DE-70 (7–10% reduction versus control) > apple DE-35 = citrus DE-35 > OPF DE-70 and LMW-D-70. Analysis of the data obtained from PEC1 revealed that the efficacy of pectin to reduce cholesterol was dependent on the pectin source, and improved with increasing MW and DE. The fact that OPP DE-70 and LMW DE-70 differed from citrus DE-70 indicates that both source and MW of pectin with a similar DE have a role.

In the subsequent PEC2 trial, citrus DE-70, an effective cholesterol-lowering pectin in PEC1, was studied again in PEC2 and compared with citrus DE-70 with a higher molecular weight. These pectins were studied at a reduced level of 6 g/day, more realistic for daily inclusion in food. At this level, citrus DE-70 and citrus HMW DE-70 similarly lowered LDL-C by about 7–9% versus control cellulose. Statistical significance was only achieved for citrus HMW DE-70. These two pectin types also lowered LDL-C/HDL-C 6–7% ($P<0.05$). The pectins studied in PEC2 did not affect the inflammatory markers hsCRP and homocysteine.

It is difficult, nevertheless tempting, to compare our study to earlier studies owing to lack of characterization of pectins and numerous other differing experimental factors. In the meta-analysis of Brown *et al*. (1999), they calculated that 1 g/day pectin reduced TC and LDL-C by −0.07 and −0.05 mmol/l respectively. In PEC1, an extrapolated dose of 1 g/day citrus DE-70 (an effective cholesterol-lowering pectin), from the actual 15-g dose, lowered these parameters less effectively, by 0.03 and 0.03 mmol/l, respectively, using pooled data from the three sub-studies ($n = 80$). In PEC2, citrus HMW DE-70 (extrapolated to 1 g/day from the actual 6 g/day) decreased LDL-C by 0.07, similar to that described by Brown *et al*. (1999).

The recently published health claim of EFSA (2010) states that 'Consumption of pectin contributes to the maintenance of normal blood cholesterol levels'. To bear the claim, information should be given to the consumer that the beneficial effect is obtained with a daily intake of at least 6 g of pectin. In addition, EFSA (2010) concludes: 'Consumption of pectin with meals contributes to the reduction of the blood glucose rise after those meals'. In order to bear this second claim, information should be given to the consumer that at least 10 g of pectin should be consumed per meal when wishing to control blood glucose levels.

Based on the present work it appears that such effects (shown for cholesterol lowering, and potentially also affecting blood glucose levels) depend on the ability of the pectin type used, to induce a viscous gastro-intestinal content, which, in turn, is shown to depend strongly on the specific molecular composition, notably DE and MW.

Generic claims for all pectin types to reduce cholesterol cannot be supported based on our data, and molecular characterization of pectin and dose should be considered for health claims.

Conflict of interest

Professor Brouns is an academic researcher and science consultant to Cargill. Dr Elke Theuwissen is an independent academic researcher at Maastricht University. Dr Adam (at time of study) was a nutrition scientist at Cargill Inc. (Vilvoorde, Belgium). Dr Bell is an independent Biostatistician at Clinical Data Services (Bloomington, IN, USA). Dr and Professor Berger is North American Nutrition Leader in Global Food Research at Cargill Inc. (Minneapolis, MN, USA). Professor Mensink is an independent academic researcher at Maastricht University.

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