Effects of *Pinus pinaster* and *Pinus koraiensis* Seed Oil Supplementation on Lipoprotein Metabolism in the Rat

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ABSTRACT: The aim of the present study was to assess the effect of vegetal oils obtained from *Pinus pinaster* and *P. koraiensis* seeds on plasma lipoprotein levels and apolipoprotein (apo) gene expression in rats. These oils contain two particular fatty acids of the Δ5-unsaturated polymethylene-interrupted fatty acid (Δ5-UPIFA) family: all-*cis*-5,9,12-18:3 (pinolenic) and/or all-*cis*-5,11,14-20:3 (sciadonic) acids. Rats were fed for 28 d a diet containing 5% (w/w) oil supplement. Two control diets were prepared to match the fatty acid composition of *P. pinaster* or *P. koraiensis* oils with the exception of Δ5-UPIFA, which were replaced by oleic acid. *Pinus pinaster* seed oil decreased serum triglycerides by 30% ($P < 0.02$), very low density lipoprotein (VLDL)-triglycerides by 40% ($P < 0.01$), and VLDL-cholesterol by 33% ($P < 0.03$). *Pinus koraiensis* seed oil decreased serum triglycerides by 16% [not statistically significant (ns)] and VLDL-triglycerides by 21% (ns). Gel permeation chromatography and nondenaturating polyacrylamide gel electrophoresis showed a tendency of high density lipoprotein to shift toward larger particles in pine seed oil-supplemented rats. Finally, *P. pinaster* seed oil treatment was associated with a small decrease of liver apoC-III ($P < 0.02$) but not in apoE, apoA-I, or apoA-II mRNA levels. The levels of circulating apo were not affected by pine seed oil supplementation. In conclusion, *P. pinaster* seed oil has a triglyceride-lowering effect in rats, an effect that is due to a reduction in circulating VLDL.

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Fatty acids have unequal lipid-lowering properties (1). Certain classes of plants, marine invertebrates, and insects contain unusual unsaturated polymethylene-interrupted fatty acids (UPIFA). Particularly, UPIFA with a *cis*-5 ethylenic bond, named Δ5-UPIFA, are characteristic and systematic components of Gymnosperm seed oils (2–4). In conifer seeds, some of the following acids may be present, depending on the botanical family considered: all-*cis*-5,9,12-18:3, all-*cis*-5,9,12,15-18:4, all-*cis*-5,11-20:2, all-*cis*-5,11,14-20:3, and all-*cis* 5,11,14,17-20:4. The lipid-lowering potential of oils containing these fatty acids is currently under investigation. *Biota orientalis* (arborvitae, a Chinese Cupressaceae) is used in traditional Chinese medicine. *Biota orientalis* seed oil decreased the concentration of serum cholesterol, high density lipoprotein (HDL)-cholesterol, and phospholipids as compared to a linoleic acid-enriched diet in hypercholesterolemic rats (5). *Pinus koraiensis* (China pine) seeds, another source of Δ5-UPIFA, are consumed in Asia as condiment for various dishes. Supplementation of cholesterol-fed rats with this oil is associated with the lowering of serum triglycerides as compared to linseed and safflower oils (6). It appears from these studies that conifer oils have different lipid-lowering potentials, which may be related in part to differences in their fatty acid composition. Therefore, a detailed analysis of the properties of conifer oils and a comparison of their effects to commercially available oils are necessary before recommending their use in the population at large. *Pinus pinaster* (maritime pine) seeds are harvested in France on a multiton scale for reforestation. The oil extracted from the seeds of this pine contains an average, 16% Δ5-UPIFA that differ in fatty acid composition from previously described pine seed oils (3).

Presently the lipid-lowering potential of *P. pinaster* seed oil is unknown. Moreover, the effect of pine seed oils on circulating lipoprotein has not been explored in detail. Therefore, the aim of our study was to assess the impact of oils extracted from *P. pinaster* and *P. koraiensis* on circulating lipoprotein fractions in rats. Since fatty acids exert part of their effect on lipoprotein levels by modulating the expression of a number of apolipoprotein (apo) genes (7), we evaluated whether any lipid-lowering effect was associated with an effect on apo gene expression and circulating levels.

EXPERIMENTAL PROCEDURES

Animals and diets. All studies were performed with 50-d-old, 200 g, male Wistar rats purchased from IFFA CREDO (L'Arbresle, France). The animals were acclimatized for 1 wk under conditions of controlled temperature (20 ± 1°C) and lighting (dark from 8 P.M. to 8 A.M.) in a room of low background noise. Rats were allowed free access to water and standard rodent chow (Rat and Mouse diet 113; UAR, Villemoisson-sur-Orge, France) during this period. Rats were then separated into

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Abbreviations: apo, apolipoprotein; FAME, fatty acid methyl ester; HDL, high density lipoprotein; ns, not statistically significant; PBS, phosphate buffered saline; UPIFA, unsaturated polymethylene-interrupted fatty acid; VLDL, very low density lipoprotein.

four groups of six rats fed *ad libitum* different diets prepared from a fat-free semipurified diet (UAR) supplemented with: (i) 5% (w/w) *P. pinaster* seed oil; (ii) 5% (w/w) of a mixture (Control-P) prepared with safflower, oleic acid-enriched sunflower, and linseed oils: 66.5, 31, and 2.5%, respectively; (iii) 5% (w/w) *P. koraiensis* seed oil; and (iv) 5% (w/w) of a mixture (Control-K) prepared with safflower, oleic acid-enriched sunflower, and linseed oils: 55, 44.7, and 0.3%, respectively. *Pinus pinaster* and *P. koraiensis* differed with respect to their fatty acid composition. *Pinus pinaster* contains less 18:1 and more 18:2 and 18:3 than *P. koraiensis*. In order to assess the potential lipid-lowering effect of Δ5 fatty acid, two control diets were prepared to match the fatty acid composition of *P. pinaster* or *P. koraiensis* and to replace the Δ5 fatty acids by oleic acid. Oleic acid was chosen because it is a monounsaturated fatty acid with properties intermediate between those of saturated and polyunsaturated fatty acids. In addition, monounsaturated fatty acids are widely recommended in the diet of dyslipidemic patients (8). The oils were provided by Society BERTIN (Lagny le Sec, France) and by R.L. Wolff for *P. pinaster*. The fatty acid compositions of these diets, as determined by gas–liquid chromatography of the methyl esters, are presented in Table 1. *Pinus pinaster* and *P. koraiensis* seed oils used in these studies contained 15.7 and 17.7%, respectively, of Δ5-UPIFA. Other dietary components were carbohydrate (63%), casein (22.5%), cellulose (6%), salt mixture (7%), and vitamin mixture (1%). Weight gain was monitored throughout the study. After 4 wk, the rats were fasted and then exsanguinated under ether anesthesia. Liver tissue and epididymal adipose tissues were removed immediately and frozen in liquid nitrogen for biochemical analysis.

Extraction of liver lipids, phospholipid isolation, fatty acid methyl ester (FAME) preparation, and analysis. Liver lipids were extracted according to Folch *et al.* (9). Phospholipids were separated by thin-layer chromatography on silica gel plates. FAME from phospholipids were prepared essentially according to Morrison and Smith (10). FAME were analyzed by gas–liquid chromatography using Varian 1400 and 940 chromatographs (Palo Alto, CA) equipped with flame-ionization detectors. Peaks were identified by comparison of their relative retention times to those of commercially available standards. Δ5-UPIFA were identified by their equivalent chain lengths according to Wolff *et al.* (11). The use of equivalent chain lengths was supported by gas–liquid chromatography–mass spectrometry of appropriate derivatives (12).

TABLE 1
Fatty Acid Composition of the Diet Fat (wt%)[a]

Fatty acids[b]	*Pinus pinaster*	Control-P	*P. koraiensis*	Control-K
16:0	3.6	5.9	4.2	5.4
16:1[c]	0.2	0.1	0.1	0.1
17:0	0.1	Trace	Trace	Trace
18:0	2.4	2.6	1.8	2.8
18:1[c]	18.1	32.7	25.5	42.3
9,12-18:2	55.9	56.2	48.4	48.1
11-20:1	1.0	0.2	1.0	0.2
9,12,15-18:3	1.3	1.5	0.2	0.2
11,14-20:2	0.8	n.d.	0.5	n.d.
Δ5 olefinic acids				
5,9-18:2	0.7	n.d.	1.8	n.d.
5,9,12-18:3	7.1	n.d.	14.9	n.d.
5,11-20:2	0.8	n.d.	0.1	n.d.
5,11,14-20:3	7.1	n.d.	0.9	n.d.
Others	0.9	0.8	0.6	0.9

[a]Fatty acid composition of the diet fat was determined by gas–liquid chromatography of the methyl esters. Results are expressed in percentage of total fat.
[b]All ethylenic bonds in the *cis* configuration.
[c]Sum of two isomers. n.d., Not detected.

Lipoprotein separation and measurements. Blood was collected from the carotid artery in dry tubes. Serum was separated by centrifugation ($630 \times g$) for 20 min at 4°C. Very low density lipoproteins (VLDL) were separated by ultracentrifugation by using a Beckman TL100 ultracentrifuge (Beckman Instruments France SA, Gagny, France), from 0.5 mL of serum by a single spin at density $1.006 \times g$ mL^{-1}. Briefly, 0.5 mL of a 0.9% NaCl solution was added to 0.5 mL of serum and spun in a polycarbonate tube ($400{,}000 \times g$, 10°C) with a Beckman TLA-100.2 rotor (Beckman Instruments France SA) for 2.5 h (13). The tube was sliced, and the remaining 0.5 mL infranate fraction ($d > 1.006$ g mL^{-1}) was analyzed for lipids. Lipids in the VLDL fraction ($d < 1.006$ g mL^{-1}) were determined by subtracting infranate values from total serum values according to the Lipid Research Clinic protocol (14).

Serum $1.019 < d < 1.21$ g mL^{-1} fraction was also separated by sequential ultracentrifugation using a Beckman TLA-100.3 rotor (Beckman Instruments France SA) at $480{,}000 \times g$ and 10°C. Two mL of serum was adjusted at $d = 1.019$ g mL^{-1} with potassium bromide and spun for 4 h. The supernate fraction was discarded, and the infranate fraction was spun at the same density for 3.5 h. Then the infranate fraction was adjusted at $d = 1.21$ g mL^{-1} and spun twice for 4 h. The resulting serum $1.019 < d < 1.21$ g mL^{-1} density fraction was dialyzed against a 10 mM phosphate buffer containing 0.01% EDTA and 0.01% sodium azide.

Lipids were determined enzymatically using commercially available kits for triglycerides (Triglycerides GPO-PAP; Boehringer Mannheim, Mannheim, Germany), cholesterol (Cholesterol C System; Boehringer Mannheim), and phospholipids (Phospholipids PAP 150; BioMérieux, Lyon, France).

Measurements of apo. Rat lipoproteins apoA-I, apoA-II, and apoE were measured by a noncompetitive enzyme-linked immunosorbent assay (sandwich ELISA). Briefly, polystyrene microtiter plates were coated with affinity-purified polyclonal antibodies to rat apoA-I, apoA-II, or apoE (1 mg/mL). Duplicate serum samples were diluted with 100 mM phosphate buffered saline (PBS) containing 1% albumin. The samples were added to the wells along with the standards and controls and incubated for 2 h at 37°C. After incubation, the plates were washed four times with PBS 100 mmol/L, and the corresponding polyclonal antibody conjugated to peroxidase was added. The plates were incubated for 2 h at 37°C and then washed. Color development was performed in 30 min by the addition of peroxidase substrate (*o*-phenylenediamine dichloride; Sigma Chemical Co., St. Louis, MO). The plates were read at 492 nm

on an automated microplate reader model EL340 (Bio-Tek Instruments, Inc., Winooski, VT). ApoC-III was measured by nephelometry using a Behring automated analyzer (Behring Nephelometer Analyser; Behring Diagnostics, Rueil Malmaison, France) and rabbit polyclonal anti apoC-III antibody.

Gel filtration chromatography. The ultracentrifugally isolated serum fraction of density $1.019 < d < 1.21$ g mL^{-1} was further separated using a Superdex 200HR 10/30 column to assess lipoprotein heterogeneity. The gel was allowed to equilibrate with PBS (10 mM) containing 0.01% EDTA and 0.01% sodium azide; 200 µL of serum was eluted with the buffer at room temperature at a flow rate of 0.2 mL min^{-1}. Elution profiles were monitored at 280 nm and recorded with an analog-recorder chart tracing system (Pharmacia LKB Biotechnology, Uppsala, Sweden). The effluents were collected in 0.24-mL fractions. Calibration was carried out with bovine albumin Fraction V (Sigma-Aldrich Chimie SARL, Saint Quentin Fallavier, France). Cholesterol was measured in each collected fraction using commercially available enzymatic kits (Cholesterol C System; Boehringer Mannheim).

Nondenaturing polyacrylamide gel and agarose gel electrophoresis. Lipoprotein sizes were identified by nondenaturing gel electrophoresis using a ready-to-use discontinuous 2–3% polyacrylamide gel LIPOFILM (Sebia, Issy-les-Moulineaux, France). Sera (10 µL) were prestained with Sudan black for 30 min at room temperature. Prestained serum aliquots (5 µL) were applied to the gel, and electrophoresis was run for 45 min at 170 V, 12 mA. The gels were scanned using an appropriate scanner (Intégrateur Lipofilm, Préférence SEBIA, HIT-HR Software 2XX, Issy-les Moulineaux, France). In order to assess whether seed oil supplementation affects lipoprotein electrophoretic mobility, agarose gel electrophoresis was performed according to Noble (15) with a Beckman Paragon system (Beckman Instruments France SA). Briefly, serum (5 mL) was applied on a 0.5% agarose gel (Paragon LIPO lipoprotein electrophoresis; Beckman Instruments France SA). Electrophoresis was performed for 30 min in a barbital buffer (pH 8.6) at 100 V. Gels were stained with Sudan black B.

RNA analysis. Total cellular RNA was isolated from liver tissue by the acid guanidinium thiocyanate/phenol–chloroform method (16). Northern and dot-blot hybridizations were performed exactly as described previously (17). The rat apoA-I, apoA-II, apoC-III, and apoE cDNA probes were described previously (18,19). All probes were labeled by random primed labeling (Boehringer Mannheim). Filters were hybridized to 1×10^6 cpm mL^{-1} of each probe as described (17). They were washed in 300 mL of 75 mM NaCl, 7.5 mM sodium citrate, pH 7.4, and 0.1% sodium dodecyl sulfate for 10 min at room temperature and twice for 30 min at 65°C and subsequently exposed to X-ray film (Kodak X-OMAT-AR; Eastman Kodak Co., Rochester, NY). Autoradiograms were analyzed by quantitative scanning densitometry (Bio-Rad GS670 Densitometer; Bio-Rad Laboratories, Richmond, CA) as described elsewhere (17).

Statistical analysis. Unpaired *t*-tests were used to compare the experimental to the control diets. Whenever a covariable (weight) explained part of the variability of the biological variable, the adjusted error term was calculated for the *t*-test using the LS-Means procedure of SAS software (SAS Institute Inc., Cary, NC).

RESULTS

The body weight, food intake, liver, and epididymal fat tissue weights were not significantly different between rats treated with *P. pinaster* or *P. koraiensis* seed oils and their respective controls (data not shown). *Pinus pinaster* and *P. koraiensis* seed oils ingestion was associated with the appearance of all-*cis*-5,11,14-20:3 and all-*cis*-5,9,12-18:3 fatty acids in liver phospholipids (Table 2). These changes were associated with a concomitant decrease of all-*cis*-9,12-18:2 and all *cis*-5,8,11,14-20:4 in *P. pinaster* seed oil-supplemented rats and with a decrease of all *cis*- 9,12-18:2 in the *P. koraiensis*-fed rats.

The effects of pine seed oil supplementation on serum lipid and lipoprotein levels were assessed after 4 wk of supplementation. The results are presented in Table 3. The levels of serum triglycerides were decreased significantly (by 30%, $P < 0.02$) in the group supplemented with *P. pinaster* seed oil compared to its respective control group. This reduction was accounted for by a decrease in the VLDL fraction, resulting in significantly lower levels of VLDL-triglycerides (–40%,

TABLE 2
Fatty Acid Composition (%) of Liver Phospholipids

Fatty acid	*P. pinaster*	Control-P	*P*[a]	*P. koraiensis*	Control-K	*P*[a]
16:0	17.6 ± 1.5	16.5 ± 1.2	ns	15.8 ± 2.4	16.8 ± 0.8	ns
9-16:1	0.8 ± 0.2	0.6 ± 0.1	ns	0.6 ± 0.2	0.7 ± 0.1	ns
18:0	21.7 ± 0.7	22.0 ± 0.6	ns	23.1 ± 2.5	22.2 ± 0.9	ns
9-18:1	2.9 ± 0.5	2.8 ± 0.1	ns	2.6 ± 0.4	3.3 ± 0.2	0.01
11-18:1	2.7 ± 00.3	2.8 ± 0.2	ns	2.7 ± 0.4	2.8 ± 0.3	ns
9,12-18:2	9.3 ± 1.0	10.5 ± 0.7	0.04	8.5 ± 0.5	9.5 ± 0.8	0.023
5,8,11,14-20:4	29.4 ± 1.1	31.6 ± 1.0	0.004	30.6 ± 1.1	31.8 ± 0.6	ns
Δ5-UPIFA						
5,9,12-18:3	0.6 ± 0.1	n.d.	0.0001	1.2 ± 0.1	n.d.	0.0001
5m11m14-20:3	1.3 ± 0.4	n.d.	0.0001	0.2 ± 0.1	n.d.	0.0008

[a]Unpaired *t*-test: *P* values for pine seed oil vs. control group. ns, not statistically significant; UPIFA, unsaturated polymethylene-interrupted fatty acid. See Table 1 for other abbreviation.

TABLE 3
Serum Lipid Concentrations (mean ± SEM) in Rats Treated with *P. pinaster* and *P. koraiensis* Seed Oils and Their Respective Controls

	P. pinaster (mmol/L)	Control-P (mmol/L)	P^a	*P. koraiensis* (mmol/L)	Control-K (mmol/L)	P^a
Cholesterol	1.86 ± 0.15	2.14 ± 0.14	ns	2.09 ± 0.16	2.06 ± 0.13	ns
Triglycerides[b]	1.24 ± 0.21	1.78 ± 0.26	0.02	1.47 ± 0.21	1.76 ± 0.21	ns
Phospholipids[b]	1.73 ± 0.10	1.87 ± 0.06	ns	1.66 ± 0.08	1.72 ± 0.06	ns
VLDL-cholesterol[b,c]	0.31 ± 0.04	0.46 ± 0.04	0.03	0.46 ± 0.08	0.49 ± 0.01	ns
VLDL-triglycerides[b,c]	0.91 ± 0.20	1.53 ± 0.26	0.01	1.21 ± 0.21	1.53 ± 0.22	ns
Cholesterol in $d > 1.006$ g mL^{-1} fraction	1.55 ± 0.14	1.68 ± 0.12	ns	1.63 ± 0.01	1.57 ± 0.12	ns

[a]Unpaired *t*-test: *P* values for pine seed oil vs. control group.
[b]Values adjusted for weight.
[c]Very low density lipoprotein (VLDL) serum $d < 1.006$ g mL^{-1} density fraction. See Tables 1 and 2 for other abbreviations.

$P < 0.01$) and VLDL-cholesterol (–33%, $P < 0.03$). Total cholesterol, cholesterol in the serum $d > 1.006$ g mL^{-1} serum density fraction, and serum phospholipid levels were not statistically significantly (ns) different between the two groups. Although there was a tendency to lower levels of serum total triglycerides (–16%, ns) and VLDL-triglycerides (–21%, ns) in rats treated with *P. koraiensis* seed oil as compared to its control group, these differences did not reach the level of statistical significance. In order to further assess the potential impact of pine seed oil supplementation on lipoprotein metabolism, the compositions of VLDL and serum $1.019 < d < 1.21$ g mL^{-1} fraction were determined (data not shown). There were no statistically significant differences in lipid composition of the various lipoproteins between treated and control rats with the exception of a moderate increase in VLDL-phospholipid ($P < 0.004$) in *P. koraiensis*-treated rats. Agarose gel electrophoretic patterns of serum lipoproteins were not different among groups (data not shown).

In order to investigate the changes in serum lipid distribution in more detail, lipoproteins were separated by gel permeation chromatography. Cholesterol was determined in the gel filtration elution fractions of the serum $1.019 < d < 1.21$ g mL^{-1} density fraction (Fig. 1). *Pinus pinaster* and *P. koraiensis* supplementation resulted in a slight shift of the HDL peak toward larger particles (Fig. 1A and B). This observation was confirmed by nondenaturing gel electrophoresis that showed a tendency, although ns, to smaller HDL relative mobility (R_f) values in the *P. pinaster* (29.8 ± 2.4 vs. 31.8 ± 2.3, ns) and *P. koraiensis* (30 ± 4 vs. 31.8 ± 4.1, ns) seed oil-supplemented rats than in controls.

Finally, we determined whether pine seed oil treatment has an effect on apo gene expression and apo levels. To this end, the liver mRNA levels of various apo were measured. The results are presented in Table 4. *Pinus pinaster* seed oil-supplemented rats had significantly lower levels of liver apoC-III mRNA than control ($P < 0.04$). This change, however, was not associated with a decrease in the concentration of serum apoC-III (*P. pinaster* 0.038 ± 0.015 vs. control 0.021 ± 0.006 g/L; ns). ApoA-I, apoA-II, and apoE liver mRNA and serum levels (A-I: 0.47 ± 0.08 vs. 0.47 ± 0.08, ns; A-II: 0.37 ± 0.10 vs. 0.45 ± 0.04, ns; E: 0.004 ± 0.003 vs. 0.002 ± 0.001, ns g/L) were not different between animals treated with *P. pinaster* seed oil and control regimen-treated rats. *Pinus koraiensis*

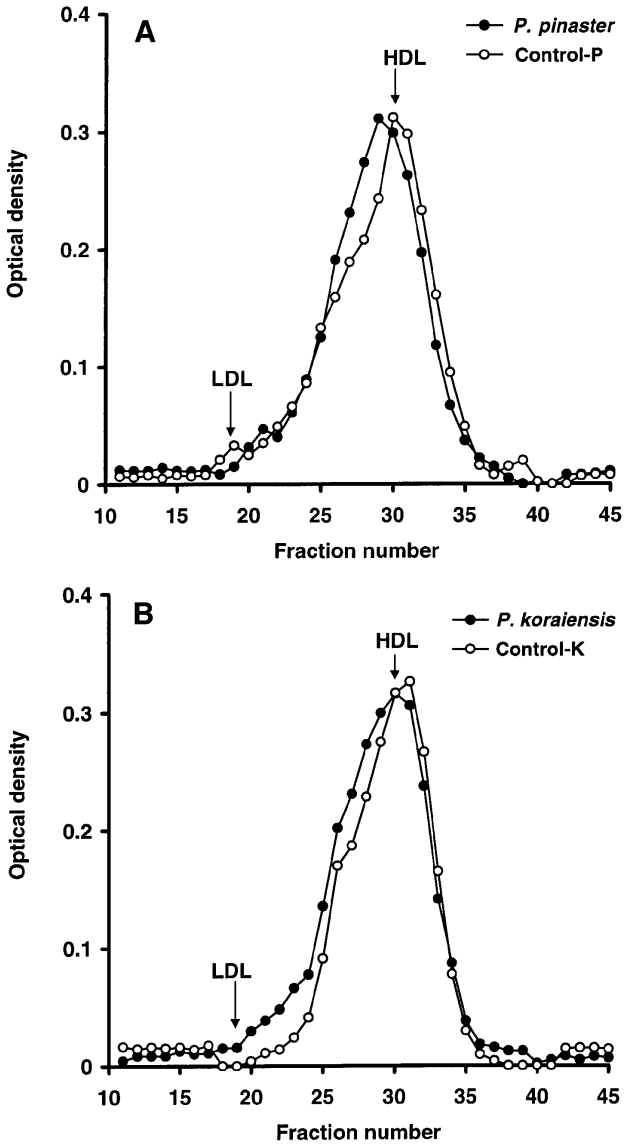


FIG. 1. Cholesterol profile of serum density fraction $1.019 < d < 1.21$ g mL^{-1} of *Pinus pinaster* (A) and *P. koraiensis* (B) supplemented rats and their respective controls. Values are medians of four profiles. The $1.019 < d < 1.21$ g mL^{-1} serum fraction was fractionated on a Superdex 200HR 10/30 column (Pharmacia LKB Biotechnology, Uppsala, Sweden). Pine seed oil and control treatments are presented with closed and open circles, respectively. HDL, high density lipoprotein; LDL, low density lipoprotein.

TABLE 4
Liver Apolipoprotein mRNA Levels (mean ± SEM) in Treated and Control Rats[a]

	P. pinaster	Control-P	P^b	*P. koraiensis*	Control-K	P^b
ApoA-I	100 ± 8	100 ± 15	ns	88 ± 9	100 ± 3	ns
ApoA-II	92 ± 3	100 ± 5	ns	105 ± 6	100 ± 5	ns
ApoC-III	85 ± 4	100 ± 5	0.04	103 ± 5	100 ± 5	ns
ApoE	100 ± 2	100 ± 6	ns	100 ± 4	100 ± 6	ns

[a]Units are percentage of controls.
[b]Unpaired *t*-test: *P* values for pine seed vs. control group; apo, apolipoprotein. See Tables 1 and 2 for other abbreviations.

seed oil supplementation had ns effect on any apo liver mRNA (apo A-I, A-II, C-III, and E) and apo levels (A-I: 0.51 ± 0.07 vs. 0.44 ± 0.07, ns; A-II: 0.37 ± 0.06 vs. 0.39 ± 0.07, ns; C-III: 0.024 ± 0.004 vs. 0.024 ± 0.005, ns; E: 0.002 ± 0.001 vs. 0.003 ± 0.003, ns g/L) compared to control diet.

DISCUSSION

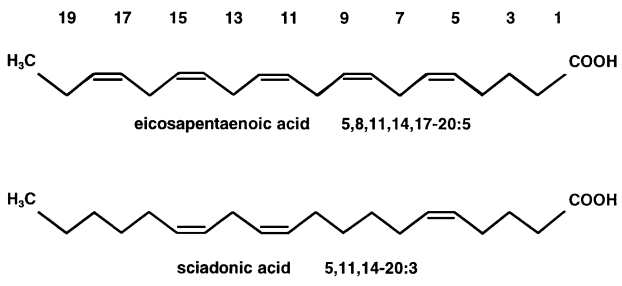
The major finding of the present study is the lowering of triglycerides, VLDL-triglycerides, and VLDL-cholesterol levels in rats treated with *P. pinaster* seed oil compared to rats treated with control regimen, in which oleic acid replaced Δ5-UPIFA. In contrast, *P. koraiensis* seed oil treatment had ns effect on lipid or on lipoprotein and apo levels compared to oleic acid-enriched diet.

Recent studies analyzed the effects of two conifer seed oils on circulating total cholesterol and triglyceride levels in rats. Ikeda *et al*. (5) found lower levels of serum cholesterol and triglycerides in hypercholesterolemic rats treated for 24 d with a diet supplemented with *B. orientalis* oil compared with a diet supplemented with linoleic acid. Similarly, Sugano *et al.* (6) observed lower levels of serum triglycerides in rats supplemented with *P. koraiensis* seed oil compared with linseed and safflower oils. In the latter studies, however, the results were confounded by the addition of 0.5% cholesterol and 0.125% sodium cholate to the diet. In the present study, all-*cis* 5,11,14-20:3 and/or all-*cis* 5,9,12-18:3 fatty acids were replaced by 18:1 in the control regimens, and no cholesterol or cholate was added to the diet, suggesting that Δ5-UPIFA has a triglyceride- and VLDL-lowering effect as compared to oleic acid. This effect is more pronounced for *P. pinaster* than for *P. koraiensis* seed oil, suggesting that sciadonic acid (all-*cis*-5,11,14-20:3), which is more abundant in *P. pinaster* seed oil, has a greater VLDL-lowering potential than pinolenic acid (all-*cis*-5,9,12-18:3). However, notably oils, not fatty acids, were used in the present study. This fact has potential implications in the interpretation of the data since factors other than fatty acids may be present in the oil and could interfere with lipoprotein metabolism.

Eicosapentaenoic acid (5,8,11,14,17-20:5) is present in marine oils and, similar to sciadonic acid (5,11,14-20:3) of *P. pinaster* seed oil, has 20 carbon atoms and *cis* double bonds in positions Δ5, Δ11, and Δ14 (Scheme 1). Eicosapentaenoic acid has a major impact on triglyceride levels (20). It is difficult to compare the results of the present study with those of previous publications on marine oils (21–24) because of differences among studies such as the amount of fat or cholesterol consumed, the length of treatment, and the type of control diet. Although the effects of *P. pinaster* seed oil on lipid and lipoprotein levels appear to resemble those of fish oils, no evidence exists that proves that *P. pinaster* oil, like fish oils, could be used safely and efficiently in man.

Clearly, evidence exists that apo have a crucial role in lipid metabolism. ApoA-I and apoA-II are key proteins of HDL metabolism (25). ApoE is necessary for lipoprotein remnant clearance (26). ApoC-III is a major component of triglyceride-rich lipoparticles and interferes with VLDL lipolysis (27–30) and uptake by cellular receptor (31–34). Evidence also exists that fatty acids exert part of their lipid-lowering effects by altering the expression of various apo genes (35). As a first indication of a possible effect of pine seed oil on apo gene expression, the mRNA levels of apoA-I, apoA-II, apoC-III, and apoE were measured in the liver of supplemented rats. Although a decreased expression of apoC-III gene in the *P. pinaster*-supplemented rats existed, this effect was modest and not associated with a decrease in serum apoC-III levels, suggesting a marginal impact of *P. pinaster* on apoC-III metabolism. Therefore, mechanisms other than a direct effect of *P. pinaster* on apo metabolism may have contributed to the triglyceride-lowering properties of *P. pinaster* seed oil, such as (i) decreased *de novo* lipid synthesis, (ii) reduced substrate availability for lipoprotein formation, or (iii) changes in VLDL physicochemical properties. Further studies are necessary to assess the mechanism of triglyceride reduction by *P. pinaster* in rats.

In the present study, *P. pinaster* seed oil supplementation reduced triglycerides and VLDL levels in rats, suggesting a potential benefit for *P. pinaster* in lowering high blood triglyceride levels. However, additional investigations in other animal models as well as controlled studies in humans are necessary before recommending the use of *P. pinaster* oil as an alternative source of oil in patients with dyslipidemia.



SCHEME 1

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