Cholesterol and Triglyceride Reduction in Rats Fed *Matthiola incana* Seed Oil Rich in (*n*−3) Fatty Acids

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Seeds of *Matthiola incana* contain oil rich (55−65%) in (*n*−3) linolenic acid. Selected lines were developed and evaluated for their agronomic and chemical parameters. Extracted oil was fed for 6 weeks to rats, which were compared with rats fed a diet containing coconut oil or sunflower oil. Cholesterol levels were significantly lowest in rats fed diets rich in *M. incana* oil (27% reduction), and triglycerides were significantly lower in rats receiving either *M. incana* or sunflower oil (36% reduction). The contents of arachidonic acid and other (*n*−6) fatty acids were significantly the lowest in the liver and plasma of rats that had received *M. incana* oil. The levels of (*n*−3) fatty acids were significantly greater in both the liver and plasma of rats fed *M. incana* oil. The ratio of (*n*−3)/(*n*−6) long-chain fatty acids in the plasma was 7 times higher in rats fed with *M. incana* oil than in those fed with sunflower oil and 6 times higher than in those fed coconut oil. The results demonstrate for the first time a beneficial effect of dietary *M. incana* oil in reducing cholesterol levels and increasing (*n*−3) fatty acid levels in the plasma. This new, terrestrial plant source of (*n*−3) fatty acids could replace marine oils and thereby contribute beneficially to the human diet.

Keywords: *Matthiola sp. seed oils; dietary (n−3) fatty acids; plasma cholesterol and triglycerides*

INTRODUCTION

Current research directions in nutrition indicate that the (*n*−3) fatty acids are receiving increasing attention as essential components of the human diet (Gibney, 1997; Lovegrove et al., 1997). The low incidence of arteriosclerosis and chronic inflammatory disease observed in the Greenland Innuit has been attributed to their traditional ethnic diet, which consists largely of marine foods rich in two (*n*−3) fatty acids: C22:6 and C20:5 (Barg and Byerberg, 1972).

Dietary fish oils containing (*n*−3) fatty acids are increasingly being recommended for their antithrombic and hypolipidemic (lowering blood lipids) effects for persons consuming typical Western diets (Phillipson et al., 1985). In addition to the potential beneficial effects of (*n*−3) polyunsaturated fatty acids (PUFA) on circulating lipids, these fatty acids have beneficial homeostatic effects that may contribute to reducing the risk of coronary heart disease; these effects include reduced platelet aggregation, reduced blood pressure and viscosity, and reduced incidence of cardiac arrhythmias (Weber and Leaf, 1991). The role of (*n*−3) PUFA in the prevention of cardiac arrhythmia has been established in animal experiments (Nair et al., 1997). Some of the mechanisms suggested by the authors include the incorporation of (*n*−3) PUFA into and modification by it of cell membrane structure, a role in eicosanoid metabolism, and, possibly, a role in cell signaling mediated through phosphoinositides.

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Most terrestrial sources of vegetable seed oil (soy, cotton, sunflower) are rich in linoleic acid and thus do not contribute to the reduction of cholesterol levels and prevention of coronary artery disease, in contrast to deep-sea fish oil (Lenz et al., 1991). However, vegetable oil enriched with (*n*−3) fatty acids could provide health benefits by avoiding any uptake of cholesterol (Hunter, 1990); therefore, a need for plant sources rich in (*n*−3) fatty acid as a replacement for or alternative to fish oil was recognized. It was shown by Budowski et al. (1984) that a linseed oil diet (rich in α-linolenic acid) affected the polyunsaturated fatty acid profile of plasma lipids in healthy volunteers. Similarly, Ishinaga et al. (1983) found that feeding rats with linseed oil altered the fatty acid composition of their platelet phospholipid, similarly to feeding them with fish oils. McLennan and Dallimore (1995) demonstrated that dietary canola oil modified myocardial fatty acids and inhibited cardiac arrythemias in rats; their results suggest that regular substitution of canola oil for other dietary lipid sources may assist in reducing the likelihood of cardiac arrhythmias. However, the effectiveness of linolenic acid is reduced by high levels of linoleic acid. Because canola oil contains only 8% (*n*−3) linolenic acid, we considered it interesting to test *Matthiola incana*, another plant from the same family (Cruciferae), a rich source of α-linolenic acid, as a potential dietary supplement.

On the basis of preliminary evaluation (Yaniv et al., 1997), we chose selected lines of *M. incana*, which is commonly used for ornamental purposes because of its colorful inflorescences but whose seeds have an average oil content of 24−25%, with (*n*−3) linolenic acid (LA) forming 55−65% of the total fatty acids in the oil (Ecker

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et al., 1992). This is among the highest known contents of LA in plant species.

We tested *M. incana* lines at our experiment station and evaluated them for potential use as a new oil crop to provide a dietary supplement by examining the effects of the seed oil in the diet on plasma cholesterol, triglycerides, and fatty acid composition of rats.

MATERIALS AND METHODS

Cultivation and Chemical Analysis of Seed Oils. Selected lines of *M. incana* with high LA contents were grown at the Bet Dagan Experiment Station during the 1993/1994 season, with each line replicated four times in a random block design. Seeds of each line were sown in 1.2 m^2 plots, each containing four rows, with 30 cm between rows, after basic fertilization consisting of 100:100:50 N:P:K had been applied at the time of soil preparation. Trifluralin (2500 g/ha) was used as a herbicide, and irrigation was applied until seedling establishment.

Observations included plant height, percentage of fertile plants, and seed yield parameters such as number of pods/plant, length of pod, number of seeds/pod, and weight of 10^3 seeds. Plants were harvested according to their dates of full maturity. Fully mature seeds from each line were oven-dried overnight at 50 °C and then analyzed for oil content and fatty acid composition (Yaniv et al., 1995).

Lipid Extraction. Seeds were dried overnight at 50 °C and ground to powder in an SEB coffee grinder (SEB, Selongey, France). Five grams of powder was mixed with 100 mL of petroleum ether (40−60 °C) (Frutarom, Haifa, Israel), and the lipid fraction was extracted in a Soxhlet apparatus (Mirom, Israel) for 16 h at 60 °C. After evaporation of solvent, the lipid fraction residues were weighed (Yaniv et al., 1991), and 0.1% Toxiquin (Sigma, Israeli Chemicals, Israel) was added to the oil designated for animal feeding to prevent oxidation. The oil was placed in 100 mL brown bottles, which were sealed under nitrogen and kept at −20 °C.

Direct Transesterification from Seeds. Seeds (200 mg) were dried overnight at 50 °C and ground into powder with a mortar and pestle, after which 0.3 mL of dichloromethane (Biolab. Co. Lab., Israel) and 2.0 mL of 0.5 mol/L sodium methoxide (MeONa) (Biolab Co. Lab.) were added. After the tube had been shaken and heated for 30 min at 50 °C, the reaction was stopped by adding 5.0 mL of water containing 0.1 mL of glacial acetic acid (Frutarom). The esterified fatty acids were extracted with 2.0 mL of petroleum ether (40−60 °C), and the clear fraction was kept at −20 °C until further analysis by injection of 2.0 μL samples into the gas chromatograph for fatty acid analysis.

Gas Chromatography of Methylated Fatty Acids. A Megabor column (DB-23, 0.5 μm film thickness, 30 m × 0.54 mm, J&W Scientific, Folsom, CA) was used in a gas chromatograph equipped with a flame ionization detector (Varian, model 3700 GC, Varian Instruments, Palo Alto, CA) and an automatic area integrator (3390A PA). The flow rate of N_2 (carrier gas) was 30 mL/min, and the oven temperature rose from 135 to 200 °C, programmed at a rate of 4 °C/min. The following fatty acids were identified by comparison with known standards (Supelco, Sigma Israeli Chemicals Ltd., Rehovot, Israel): C16:0, palmitic; C18:0, stearic; C18:1, oleic; C18:2, linoleic; C18:3, linolenic; C20:1, eicosenoic; C22:1, erucic acid; C20:4, arachidonic; C20:5, eicosapentaenoic: C24:1, nervonic.

Feeding Experiment. *Animals and Diet.* Twenty-seven Sprague-Dawley (Harlen, Israel) male rats, weighing 175−205 g, housed individually in suspended stainless steel cages, were maintained at 22−24 °C on a 12-h light−dark cycle with free access to food and water in accordance with guidelines issued by The Hebrew University of Jerusalem, Israel. Rats were assigned randomly to one of three groups and fed one of the following diets: *M. incana* oil (MTO), sunflower oil (SO), or coconut oil (CO). Toxiquin, at a concentration of 0.1 g/100 g, was added to all of the oils (Table 1).

Table 1. Composition of the Three Diets Used in the Experiment

	diet (g/kg)		
ingredient	CO	MTO	SO
casein	200	200	200
methionine	3	3	3
corn starch	304	304	304
sucrose	198	198	198
cellulose	50	50	50
coconut oil	200		
Matthiola oil		200	
sunflower oil			200
toxiquin[a]	0.2	0.2	0.2
salt mix[b]	35	35	35
vitamin mix[c]	10	10	10
choline	2	2	2

[a] 100 mg/100 g of oil. [b] Mineral mix AIN 76A (AIN 1977, 1980). [c] Vitamin mix AIN 76A (AIN 1977, 1980).

Table 2. Fatty Acid Composition of the Lipids Used for the Diet Formulation

	fatty acids in oil (g/100 g)		
fatty acid	coconut	*M. incana*	sunflower
short-chain (C4−C10)	13.7		
lauric acid (C12:0)	45.7		
myristic acid (C14:0)	16.1		
palmitic acid (C16:0)	8	8.6	7
stearic acid (C18:0)	2.6	2.5	3.3
oleic acid [C18:1(*n*−9)]	10.3	15.1	19.6
linoleic acid [C18:2(*n*−6)]	3.8	14.4	69.1
linolenic acid [C18:3(*n*−3)]		59.4	1

The fatty acid compositions of the lipids used for diet formulation appear in Table 2.

The dry ingredients of the diets were mixed and frozen at −18 °C, and the quantity needed was mixed with the oils every 2 days to provide the animals with fresh food. Food intake was measured every 2 days, and body weight was recorded once a week for 6 weeks.

Blood and Tissue Collection Procedure. Following an overnight food deprivation, blood samples (8−10 mL) were obtained through the vena cava, collected in polyethylene tubes that had been prewashed with heparin (4×10^5 units/L) (Sigma Chemical Co., St. Louis, MO), and dried overnight. The samples were centrifuged for 4−5 min at 10000*g*, and the plasma was removed and frozen at −20 °C for further use. After 6 weeks of consuming the diets, the rats were anesthetized with Nembutal (Ceva, France) using 0.2 mL/100 g of body weight. The tissues, including the liver, were excised and weighed, and the liver was immediately frozen with liquid nitrogen and kept at −70 °C until further analysis.

Determination of Plasma Cholesterol and Triglyceride Levels. Plasma cholesterol and triglyceride levels were determined enzymatically with commercial kits (Raichem, San Diego, CA) according to the methods of Allain et al. (1974) and McGowan et al. (1983), respectively.

Determination of Plasma Fatty Acid Composition. The lipid fraction was extracted from the plasma according to the method of Folch et al. (1957). One humdred microliters of plasma was mixed with 2 mL of extraction solution containing chloroform/methanol, 2:1 (Frutarom) and shaken well. After 30 min, 1 mL of distilled water was added to the extraction mixture, which was shaken well and left until phase separation, when the chloroform lower phase was collected with a Pasteur pipet and dried under nitrogen.

Methylation of Fatty Acids. A amount of 2.5 mL of 5 mol/L H_2SO_4 (BDH Chemicals Ltd., England) in methanol solution was added to the lipid fraction. The tube was then closed, shaken well, and placed in a 65 °C water bath for 1 h. The methylated fatty acids were extracted by adding of 1 mL of petroleum ether (40−60 °C) and 2 mL of distilled water, shaking well, and then collecting the petroleum ether upper

Table 3. Yield and Oil Parameters of *M. incana* Cultivated at Bet Dagan

		ROZ 19[a]	ROZ 45[a]
plant	height (cm)	$164.0^{a} \pm 6.9$	$139.8^{b} \pm 14.7$
	fertile (%)	$89.8^{a} \pm 1.7$	$86.3^{a} \pm 3.7$
pods	number/plant	$79.0^{a} \pm 3.9$	$65.5^{b} \pm 13.5$
	length (cm)	$7.8^{b} \pm 0.2$	$10.0^{a} \pm 0.5$
seeds	number/pod	$57.8^{b} \pm 1.3$	$62.8^{a} \pm 0.8$
	1000 seed wt (g)	$2.0^{b} \pm 0.1$	$2.4^{a} \pm 0.1$
	yield (kg/ha)	$780^{a} \pm 90$	$675^{ab} \pm 25$
oil[b]	%	$24.7^{a} \pm 0.54$	$25.3^{a} \pm 0.17$
C18:3[c]	%	$56.1^{ab} \pm 0.44$	$57.1^{a} \pm 0.59$

[a] The numbers are means ± SEM for four replicates. Means not sharing a common superscript letter are significantly different at $p < 0.001$. [b] Percent oil of dry seed weight. [c] Percent C18:3 of total fatty acids in oil.

phase, which contains the fatty acid methyl esters, with a Pasteur pipet.

Gas chromatography of fatty acids was as described for seed analysis.

Determination of Liver Cholesterol. Liver cholesterol was measured according to the method of Searcy and Bergquest (1960) with the following modifications: 40−100 mg samples of liver were saponified with 3 mL of 10 mol/L KOH (Frutarom) in ethanol for 1 h at 65 °C in a water bath, and 3 mL of distilled water was added to the cooled mixture. This was followed by a series of three successive extractions into 5, 3, and 3 mL of petroleum ether, and the extractions were pooled and dried under nitrogen at 60 °C in a water bath. The dried cholesterol was dissolved in 1 mL of petroleum ether, and 200-μL samples were taken and dried under nitrogen. A calorimetric reaction was then carried out in 3 mL of glacial acetic acid saturated with $FeSO_4$ (Frutarom) and 1 mL of concentrated H_2SO_4, and the results were read at 490 nm on a Gilford spectrophotometer (model 2400, Gilford, CA).

Determination of Liver Fatty Acid Composition. The fraction left after the extractions was neutralized with 5 mol/L HCl (Frutarom), after the addition of three drops of methyl red (ACS Reagent Sigma, Rehovot, Israel), the fatty acids were extracted into 2 mL of petroleum ether, and the extract was subsequently dried under nitrogen. The fatty acids were methylated with a methylation mixture containing 5 mol/L H_2SO_4 in methanol. Five milliliters of the methylation mixture was added to the tube, which was then heated for 1 h in a 65 °C water bath. The esterified fatty acids were extracted after the addition of 3 mL of distilled water and 2 mL of petroleum ether, and the fatty acid composition was determined by GC as described above.

Statistical Analysis. Agronomical data and feeding experiments were statistically analyzed using ANOVA unilateral variance test and Duncan's multiple-range test (SAS/STAT version 6, SAS Institute, Cary, NC). Differences were considered significant when p was <0.001.

RESULTS AND DISCUSSION

Agronomy. Table 3 summarizes the results of the evaluation experiment of two lines of *M. incana* cultivated at Bet Dagan from autumn 1993 to spring 1994. The calculated yield was 675−780 kg of seeds/ha, with an oil concentration between 24.7 and 25.3 g/100 g of the dry seed weight and an estimated yield of 180 L of oil/ha. A 56−57% content of LA ($n-3$) in the oil would yield ∼100 L of pure LA from 1 ha of the crop. With such an oil yield, *M. incana* lines represent a potential oil seed crop for dietary purposes.

Feeding Experiments. *(a) Body Weight.* No significant differences in body weight among the various dietary groups were apparent during the experiment.

Table 4. Effects of the Various Plant Oil Diets, Fed for 6 Weeks, on Weight Gain, Food Intake, and Liver Weight of Rats[a]

oil in diet	wt gain (g/6 weeks)	food intake (g/day)	liver wt (g/100 g of body wt)
coconut	210 ± 12	19.1 ± 0.6	2.60 ± 0.06^{b}
Matthiola	229 ± 11	18.8 ± 0.6	2.94 ± 0.11^{a}
sunflower	232 ± 10	18.1 ± 0.4	2.78 ± 0.05^{ab}

[a] Results are means ± SEM, $n = 9$. Means not sharing a common superscript letter are significantly different at $p < 0.05$.

Table 5. Effects of the Various Plant Oil Diets on Plasma Cholesterol, Plasma Triglycerides, and Liver Cholesterol in Rats[a]

oil diet	plasma cholesterol (mmol/L)	plasma triglycerides (mmol/L)	liver cholesterol (mmol/g)
coconut	69.3 ± 6.2^{a}	80.7 ± 10.2^{a}	3.94 ± 0.27^{b}
M. incana	50.7 ± 4.8^{b}	53.5 ± 4.3^{b}	5.53 ± 0.27^{ab}
sunflower	63.7 ± 4.2^{ab}	53.7 ± 4.2^{b}	6.80 ± 0.7^{a}

[a] Results are means ± SEM, $n = 9$. Means not sharing a common superscript letter are significantly different at $p < 0.05$.

The *M. incana* oil diet did not influence final weight, weight gain, or food intake, as shown in Table 4. Our results are in agreement with previous findings for various oil sources rich in ($n-3$), which also demonstrated no effect on body weight (Sheppard and Herzberg, 1992). The fact that the diets did not affect body weight is important, because this is the first time that *M. incana* oil has been tested in a feeding experiment and it indicates its potential as a dietary oil. In addition, we observed no indications of toxicity.

(b) Plasma Cholesterol, Triglycerides, and Liver Cholesterol. Plasma cholesterol concentrations were lower in rats fed the *M. incana* oil diet than in the rats receiving other oils (Table 5).

A reduction in human plasma cholesterol as a result of a diet rich in α-linolenic acid was demonstrated by Indu and Ghafoorunissa (1992) and Cunnane et al. (1993), and comparisons between linoleic and linolenic acids showed that both induced a reduction in cholesterol (Chan et al., 1993; Singer, 1992).

A previous study showed that replacement of coconut oil with oil rich in unsaturated fatty acids (corn, sunflower) resulted in a decrease in plasma cholesterol levels (Kinsella et al., 1990), which was attributed to reduction in very low density lipoprotein−trigyceride synthesis and improvement in low density lipoprotein cholesterol clearance.

Liver cholesterol levels of rats fed coconut oil were significantly lower than those of rats fed sunflower oil, whereas rats fed *M. incana* oil reached medium cholesterol levels, not significantly different from those of rats fed the other diets. Teik and Tan (1992) reported similar findings in experiments conducted on hamsters fed various oils. Liver cholesterol was lower in coconut-oil-fed hamsters than in those receiving other oils, but their heart cholesterol level was higher. This could have been due to altered partitioning of cholesterol among the various animal tissues as a result of the diet (Teik and Tan, 1992). Plasma triglyceride levels were significantly ($p < 0.05$) lower in rats fed either *M. incana* or sunflower oil than in those fed coconut oil (Table 5), similar to the findings reported by Chan et al. (1991) for healthy humans fed a diet rich in α-linolenic acid. Rustan et al. (1992) showed that plasma triglyceride

Table 6. Effects of the Various Plant Oil Diets on Plasma Fatty Acid Composition in Rats Fed for 6 Weeks

	g/100 g of total fatty acids[a]		
fatty acid	coconut	*Matthiola*	sunflower
lauric acid (C12:0)	1.6 ± 0.7[a]	0.5 ± 0.5[a]	0.3 ± 0.3[a]
myristic acid (C14:0)	5.4 ± 0.2[a]	1.1 ± 1.1[b]	1.7 ± 1.0[b]
palmitic acid (C16:0)	19.9 ± 0.7[a]	18.8 ± 0.5[b]	15.9 ± 0.3[b]
palmitoleic acid [C16:1(*n*−7)]	2.4 ± 0.3[a]	0.4 ± 0.2[b]	0.4 ± 0.2[b]
stearic acid (C18:0)	11.7 ± 1.1[a]	13.9 ± 1.3[a]	13.0 ± 0.6[a]
oleic acid [C18:1(*n*−9)]	18.0 ± 0.9[a]	11.0 ± 0.8[b]	9.1 ± 0.5[b]
linoleic acid [C18:2(*n*−6)]	14.4 ± 0.6[c]	20.4 ± 1.7[b]	27.4 ± 1.5[a]
linolenic acid [C18:3(*n*−3)]	1.1 ± 0.4[b]	15.7 ± 1.6[a]	0.6 ± 0.2[b]
eicosatrienoic acid (C20:3)	0.6 ± 0.3[a]	0.6 ± 0.2[a]	nd[b]
arachidonic acid [C20:4(*n*−6)]	20.4 ± 1.5[b]	11.3 ± 1.3[c]	29.5 ± 1.0[a]
icosapentaenoic acid [C20:5(*n*−3)]	nd	4.4 ± 0.5	nd
nervonic acid [C24:1(*n*−9)]	0.6 ± 0.3[a]	0.3 ± 0.2[a]	0.9 ± 0.5[a]
docopentaenoic acid [C22:5(*n*−3)]	nd	0.7 ± 0.4	nd
docosahexaenoic acid [C22:6(*n*−3)]	2.1 ± 0.3[a]	2.0 ± 0.4[a]	0.3 ± 0.2[b]
others	2.0 ± 0.7[a]	1.1 ± 0.4[a]	1.1 ± 0.2[a]
total (*n*−6)[c]	20.4 ± 1.5[b]	11.3 ± 1.3[c]	29.5 ± 1.0[a]
total (*n*−3)[c]	2.1 ± 0.3[b]	7.0 ± 0.7[a]	0.3 ± 0.2[c]
(*n*−3)/(*n*−6)	0.11 ± 0.01[b]	0.63 ± 0.03[a]	0.01 ± 0.005[c]

[a] Results are means ± SEM, $n = 4$. Means not sharing a common superscript letter are significantly different at $p < 0.05$. [b] Not detected. [c] Only fatty acid with 20 carbons or more.

Table 7. Effects of the Various Plant Oil Diets on Liver Fatty Acid Composition in Rats Fed for 6 Weeks

	g/100 g of total fatty acids[a]		
fatty acid	coconut	*Matthiola*	sunflower
lauric acid (C12:0)	1.8 ± 0.2	nd[b]	nd
myristic acid (C14:0)	2.9 ± 0.2[a]	0.3 ± 0.1[b]	0.3 ± 0.1[b]
palmitic acid (C16:0)	21.8 ± 0.7[a]	14.7 ± 0.3[b]	14.6 ± 0.3[b]
palmitoleic acid [C16:1(*n*−7)]	1.3 ± 0.3[a]	0.3 ± 0.1[b]	0.2 ± 0.1[b]
stearic acid (C18:0)	15.1 ± 0.8[a]	11.9 ± 1.1[a]	11.5 ± 1.1[a]
oleic acid [C18:1(*n*−9)]	21.5 ± 0.7[a]	12.8 ± 0.6[b]	12.2 ± 0.6[b]
linoleic acid [C18:2(*n*−6)]	12.1 ± 0.6[a]	18.9 ± 0.5[b]	38.0 ± 2.0[a]
δ-linolenic acid [C18:3(*n*−6)]	nd	nd	0.6 ± 0.1
α-linolenic acid [C18:3(*n*−3)]	0.2 ± 0.1[b]	21.0 ± 1.5[a]	0.3 ± 0.1[b]
eicosatrienoic acid (C20:3)	1.0 ± 0.4[a]	0.3 ± 0.1[a]	0.3 ± 0.1[a]
aracidonic acid [C20:4(*n*−6)]	18.3 ± 0.8[a]	7.7 ± 0.8[b]	17.5 ± 1.1[a]
icosapentaenoic acid [C20:5(*n*−3)]	nd	4.4 ± 0.1	nd
nervonic acid [C24:1(*n*−9)]	0.7 ± 0.3[a]	nd	1.2 ± 0.3
docopentaenoic acid [C22:5(*n*−3)]	nd	2.7 ± 0.1	nd
docosahexaenoic acid [C22:6(*n*−3)]	3.2 ± 0.2[b]	4.5 ± 0.3[a]	1.6 ± 0.2[c]
others	0.2 ± 0.1[b]	0.7 ± 0.2[a]	0.7 ± 0.1[a]
total (*n*−6)[c]	18.3 ± 0.8[a]	7.7 ± 0.8[b]	18.6 ± 1.1[a]
total (*n*−3)[c]	3.2 ± 0.2[b]	11.6 ± 0.5[a]	1.6 ± 0.2[c]
(*n*−3)/(*n*−6)	0.18 ± 0.01[b]	1.63 ± 0.18[a]	0.08 ± 0.01[c]

[a] Results are means ± SEM, $n = 9$. Means not sharing a common superscript letter are significantly different at $p < 0.05$. [b] Not detected. [c] Only fatty acid with 20 carbons or more.

levels were lower in rats fed a diet containing 20% flax, sunflower, or fish oil than in those receiving other oils; the response to fish oil was the greatest. Coniglio (1992) suggested that the decrease in plasma triacylglycerol caused by dietary fish oils is somehow related to a decrease in the capacity of the liver to hydrolyze phosphatidate, which then affects microsomal synthesis of triacylglycerol from diacylglycerol. Wong et al. (1984) demonstrated in their studies that the lowering of plasma triacylglycerols by fish oil reflects diminished lipogenesis, increased fatty acid oxidation, possibly in peroxisomes, and diminished secretion of triacylglycerols by the liver.

(c) Plasma and Liver Fatty Acid Composition. The differences observed in the fatty acid composition of the plasma and the liver in response to various dietary fats are summarized in Tables 6 and 7, respectively. The fatty acid compositions of the plasma and liver lipids varied with the type of dietary fat: the coconut oil diet resulted in the highest levels of saturated fatty acids (C12:0, C14:0; C16:0) and also of the monounsaturated fatty acids C16:1 and C18:1, in both plasma and liver, whereas the levels of linoleic acid [C18:2(*n*−6)] were higher in the plasma and liver of sunflower-oil-fed animals. The *M. incana* oil diet resulted in higher levels of α-linolenic acid [(C18:3(*n*−6)] in both plasma and liver.

The level of arachidonic acid [C20:4(*n*−6)] dropped significantly ($p < 0.01$) as a result of the *M. incana* oil diet, whereas two (*n*−3) fatty acids appeared only after administration of this diet: C20:5 eicosapentaenoic acid (EPA) and C22:5 docosahexaenoic acid (DHA) (*n*−3). A similar observation was reported by Halvorsen et al. (1995), who fed rats with concentrated fish oil fractions consisting of monounsaturated fatty acids or (*n*−3) polyunsaturated fatty acids. The mechanism for the reduction of the arachidonic acid level is not known, but Halvorsen et al. (1995) suggested that an inhibition of C18:2(*n*−6) interconversion to C20:4(*n*−6) by C20:5(*n*−3) and other long-chain fatty acids obtained from fish might be an important factor behind the decrease in C20:4(*n*−6) levels.

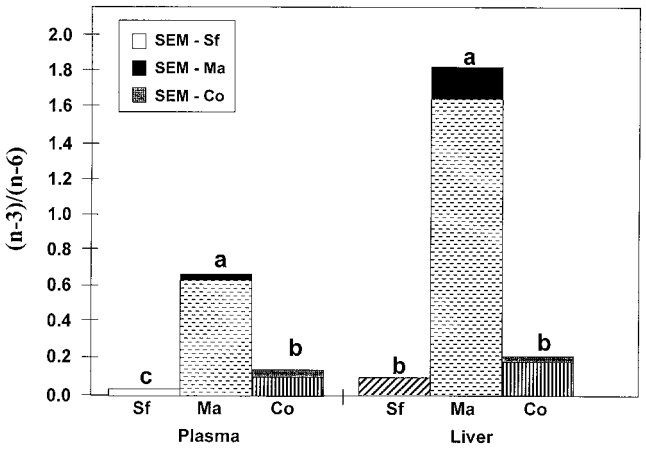

Figure 1. Plasma and liver ($n-3$)/($n-6$) ratio in rats. Results are means ± SEM, $n = 9$. Values with different letters differ significantly at $p < 0.05$.

In the present study, there was also a slight rise in the levels of C22:5($n-3$), especially in the liver, as a result of which the sum of long-chain fatty acids of the ($n-6$) family dropped after the *M. incana* oil diet (Tables 6 and 7), whereas that of those of the ($n-3$) family rose (Tables 6 and 7), resulting in much higher ($n-3$)/($n-6$) ratios in both plasma and liver (Figure 1).

(d) (n−3)/(n−6) in Plasma and Liver. One of the major goals of the present work was to test the effect of an *M. incana* oil diet, rich in α-linolenic acid, on the concentration of long-chain ($n-3$) fatty acids in plasma and liver. As shown in Tables 6 and 7 and Figure 1, the *M. incana* diet resulted in a higher level of ($n-3$) fatty acids in both liver and plasma, as compared with the other oil diets (coconut and sunflower). EPA was high in both liver and plasma; C22:5($n-3$) and DHA were high in both, with higher values in the liver. Similar results were obtained by Ishinaga et al. (1983), who used flax and sunflower oil diets.

In an effort to evaluate which dietary fat might provide the best response in terms of plasma lipid and lipoprotein levels, Sirtori et al. (1992) fed human patients corn oil, olive oil, or a supplement of ($n-3$) fatty acids. The results indicated that the ($n-3$) fatty acid diet lowered total cholesterol and triglyceridemia significantly; similar to the results of our present studies, the three regimens studied by Sirtori et al. (1992) altered the fatty acid profiles of plasma and cell lipids in a way reflecting the fatty acid composition of the administered fats.

This work strongly supports our belief that using *M. incana* oil as a supplement to the diet could offer an excellent alternative to fish oil or any other ($n-3$) fatty acid in providing a beneficial effect of lowering plasma lipids and triglycerides.

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