

## Comparative effects of rose hip and corn oils on biliary and plasma lipids in rats<sup>1</sup>

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**SUMMARY.** The comparative effects of dietary level and time of feeding corn (CO) and rose hip (RHO) oils on bile and plasma lipid composition were studied. 48 males Sprague Dawley rats were divided in two groups fed semipurified diets containing CO or RHO as the only lipid source. Groups of 6 rats were fed ad libitum diets containing 5% or 15% vegetable oil during 15 or 60 days. Food intake was not dependent on the type of oil, and was higher in 15% oil diets ( $p < 0.01$ ), increasing with time of feeding ( $p < 0.001$ ). Bile flow was similar in all groups. Biliary concentrations of cholesterol, phospholipids and bile acids were affected by the time of feeding ( $p < 0.001$ ). Plasma total and high density lipoprotein (HDL) cholesterol levels were higher in 15% oil fed rats ( $p < 0.05$ ). Triglycerides concentrations were similar in all groups. The results indicate that oil concentration and time of feeding were the most important variables affecting the lipid composition of rats, independently of the fatty acid composition of the ingested fats.

**RESUMEN.** Efectos comparativos de la ingesta de aceites de maíz y rosa mosqueta sobre la composición de lípidos biliares y plasmáticos en ratas. En el presente trabajo se estudiaron los efectos comparativos de la concentración y tiempo de ingesta de aceites de

maíz (CO) y rosa mosqueta (RHO) sobre la composición de lípidos biliares y plasmáticos de ratas. 48 ratas macho *Sprague Dawley* fueron divididas en dos grupos alimentados con dietas semipurificadas elaboradas con CO o RHO como única fuente lipídica. Grupos de 6 ratas se alimentaron durante 15 ó 60 días con dietas que contenían 5% ó 15% (p/p) de los aceites vegetales en ensayo. La ingesta dietaria fue independiente del tipo de aceite ingerido, y fue mayor en las ratas que ingirieron aceites al 15% ( $p < 0.01$ ), aumentando con el tiempo de alimentación ( $p < 0.001$ ). El flujo biliar fue semejante en todos los grupos experimentales. Las concentraciones biliares de colesterol, fosfolípidos y ácidos biliares fueron afectadas por el tiempo de ingesta ( $p < 0.001$ ). La concentración plasmática de colesterol total y unido a lipoproteínas de alta densidad (HDL) fueron más altas en las ratas que ingirieron dietas que contenían 15% de aceite ( $p < 0.05$ ). Las concentraciones plasmáticas de triglicéridos fueron similares en todos los grupos de animales. Los resultados indican que las variables concentración de grasa dietaria y tiempo de ingesta fueron más importantes que la composición en ácidos grasos de los aceites vegetales ingeridos sobre los parámetros estudiados.

### INTRODUCTION

A promotion of the consumption of vegetable oils rich in linoleic (18:2 n-6) and linolenic (18:3 n-3) acids has been observed in the last decade, due to their hypocholesterolemic effect (1,2). This action has been especially highlighted by vegetable oil producers, who also point to the fact that this is

a cholesterol-free dietary fat source.

Corn (*Zea mais*) oil is an edible fat widely used, with linoleic acid reaching nearly 50% its glycerides content. Rose hip (*Rosa moschata Mill*) seed oil is not currently accepted as an edible fat. It presents an extremely high linolenic acid content, near 28%, which induces its rapid oxidation.

In previous experiments, we observed a plasma cholesterol lowering effect of rose hip oil, without measurable change in biliary lipids output (3). Under different experimental conditions, feeding this oil increased biliary concentration of cholesterol (4). The aim of this work was to investigate whether rat plasma and biliary lipid concentrations are related to the type of polyunsaturated oil ingested, its amount in diet and/or period of intake.

Groups of rats were fed diets varying in fat type (corn or

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rose hip oils), dietary fat level (5% or 15%) and length of treatment (15 or 60 days). Data on the effects of these variables upon biliary and plasma lipid concentrations are presented.

## METHODS AND MATERIALS

### Animal and diets

Forty-eight healthy male Sprague Dawley rats weighing  $93 \pm 20$  g were used in this study. Animals were randomly divided into two groups (24 per treatment). One group was fed corn oil, CO, (Mazola) or rose hip oil, RHO, (COESAM) as the only lipid source. The fatty acid composition of oils is shown in Table 1 and the composition of experimental diets is given in Table 2. Rats were fed CO or RHO at 5% (wt/wt) or 15% (wt/wt) level in the diet. Rats were housed individually in wire bottomed cages and kept under controlled temperature and relative humidity conditions, and 12 h light-dark cycles. Animals had free access to food and water.

After 15 or 60 days of dietary treatment, 20 h fasting rats ( $n=6$  per group) were anaesthetized with sodium pentobarbital (60 mg/kg body weight, intraperitoneally).

TABLE 1  
RELATIVE FATTY ACID COMPOSITION  
OF CORN AND ROSE HIP OILS

Fatty acid	g/100g	
	Corn oil	Rose hip oil
14:0	—	0.1
16:0	9.0	3.7
18:0	1.1	1.0
18:1	28.9	17.6
18:2	59.5	49.8
18:3	1.5	27.8

TABLE 2  
COMPOSITION OF EXPERIMENTAL DIETS

Ingredient	Amount	
	Low fat diet	High fat diet
	g/100g	
Casein	20.0	20.0
DL-Methionine	0.3	0.3
Mineral mixture <sup>1</sup>	4.0	4.0
Vitamin mixture <sup>1</sup>	1.0	1.0
Cellulose	5.0	5.0
Corn starch	19.7	19.7
Sucrose	45.0	35.0
Vegetable oil <sup>2</sup>	5.0	15.0

<sup>1</sup> AIN-76 (American Institute of Nutrition. Report of the AIN ad hoc Committee on Standards for Nutritional Studies. J. Nutr. 107:1340-1348, 1977).

<sup>2</sup> Corn oil or Rose hip oil

### Bile and blood sampling

Bile duct was cannulated (PE-10 tubing) and bile was collected in preweighed Eppendorf tubes every 15 min for 60 min. Blood was collected from the abdominal aorta. All samples were frozen ( $-20^{\circ}\text{C}$ ) until assayed.

### Analysis

Biliary and plasma cholesterol concentrations were determined using commercially available enzymatic kits. Plasma and biliary concentrations of total cholesterol were determined using Merckotest 14366 enzymatic UV test. Plasma high density lipoprotein (HDL) cholesterol was measured using the same assay after the precipitation of low density lipoprotein (LDL) and very low density lipoprotein (VLDL) cholesterol fractions with heparin and magnesium ions, removed by centrifugation at  $1500 \times g$  for 30 min. LDL and VLDL cholesterol concentrations were estimated by the formula of Friedewald et al (5). Triglyceride concentrations in plasma were determined using Merckotest 14354 enzymatic colorimetric test. Biliary phospholipids were assayed by the colorimetric quantitation of phosphorus (6). Bile acids were measured by the method of Talalay (7), modified (8).

### Statistical analysis

All data are expressed as a mean value  $\pm$  standard deviation. Results were analyzed by two-way analysis of variance (ANOVA). A probability value ( $p$ ) of 0.05 or less was considered statistically significant.

## RESULTS

Food intake and body growth of rats fed diets containing 5% or 15% vegetable oils are shown in Table 3. Food ingestion was not dependent on the nature of dietary oil. Rats consumed higher amounts of 15% oil diets ( $p < 0.001$ ) increasing food intake with time ( $p < 0.001$ ). Rats fed 15% oil gained significantly more weight in all periods assayed. Food utilization (efficiency) decreased with time, and was higher in 15% oil diets.

By the end of the experiments, liver weight was similar in the CO and RHO groups, and was affected by oil quantity, as 15% oil fed rats showed larger livers ( $p < 0.001$ ). Feeding period was negatively related to relative liver weight ( $p < 0.001$ ): it decreased with time as the animals grew.

Bile flow was similar in CO and RHO groups. No significant difference was observed in any of the oil amounts and feeding periods assayed. This result and the biliary concentrations of cholesterol, phospholipids and bile acids are presented in Table 4. The concentration of biliary cholesterol was not dependent on the type of vegetable oil ingested. During the first 15 days of dietary treatment, higher concentration of cholesterol were measured in CO and RHO groups ( $p < 0.001$ ). The intake of both vegetable oils caused a decrease in biliary concentration of phospholipids and bile acids with time ( $p < 0.001$ ). Phospholipid levels were always lower in CO group, while animals fed RHO exhibited lower bile acids concentration at the same feeding periods.

TABLE 3  
FOOD INTAKE, WEIGHT GAIN AND RELATIVE LIVER WEIGHT OF RATS FED THE EXPERIMENTAL DIETS FOR 15 OR 60 DAYS<sup>1</sup>

Dietary fat Type	Time %	Days	Food intake g	Weight gain g	Relative liver weight %
Corn oil	5	15	198.3±23.4	77.1±14.0	4.3±0.7
		60	908.8±118.7	267.3±43.3	3.7±0.7
Corn oil	15	15	232.0±20.4	92.2±15.3	4.8±0.1
		60	964.7±82.6	289.0±43.3	3.7±0.2
Rose hip oil	5	15	222.2±30.3	83.50±17.1	4.1±0.3
		60	914.4±107.5	265.2±52.8	4.0±0.4
Rose hip oil	15	15	240.9±12.0	100.8±13.4	5.1±0.5
		60	1012.9±81.9	299.3±25.3	3.9±0.4

<sup>1</sup> Values are mean±S.D. (n=6). Significant effects of variables were evaluated with two-way analysis of variance (ANOVA). Significance levels are indicated under columns as follows:

Type of oil	: NS	NS	NS
Concentration:	: p<0.01	p<0.01	p<0.01
Days of intake	: p<0.001	p<0.001	p<0.001

TABLE 4  
BILE FLOW AND BILIARY LIPID CONCENTRATIONS OF RATS FED THE EXPERIMENTAL DIETS FOR 15 OR 60 DAYS<sup>1</sup>

Dietary fat Type	Time %	Days	Bile flow uL/g liver/min	Cholesterol mmol/L	Phospho-lipids mmol/L	Bile acids mmol/L
Corn oil	5	15	1.22±0.23	1.15±0.34	3.67±2.20	26.32±8.67
		60	1.36±0.13	0.33±0.07	1.65±0.39	16.85±5.76
Corn oil	15	15	1.51±0.21	0.68±0.13	2.96±0.98	29.74±5.45
		60	1.32±0.30	0.43±0.09	1.53±0.19	17.26±4.42
Rose hip oil	5	15	1.29±0.13	0.72±0.26	3.81±1.20	23.11±4.41
		60	1.37±0.34	0.27±0.02	1.85±0.36	19.17±3.87
Rose hip oil	15	15	1.28±0.14	0.85±0.14	3.14±0.35	25.71±6.96
		60	1.40±0.13	0.43±0.06	1.97±0.39	14.51±5.00

<sup>1</sup> Values are mean±S.D. (n=6). Significant effects of variables were evaluated with two-way analysis of variance (ANOVA). Significance levels are indicated under columns as follows:

Type of oil :	NS	NS	NS	NS
Concentration :	NS	NS	NS	p<0.05
Days of intake :	NS	p<0.01	p<0.01	p<0.01

An elevated cholesterol/phospholipids (C/PL) ratio is considered as a lithogenic index, due to the increase in biliary cholesterol output and/or relative decrease in phospholipids output. C/PL ratio exhibited higher values in 15% oil fed rats, and was not affected by the feeding period of the experimental diets.

Plasma concentrations of cholesterol and triglycerides were not affected by the type of oil ingested or the period of feeding. Data of these parameters are shown in Table 5. The oil content of the experimental diets influenced plasmatic cholesterol level, which was lower in rats fed 15% oil diets (p<0.05). Rats fed 5% RHO exhibited lower cholesterol concentration than 5% CO group (p<0.05) and decreased with time of feeding (p<0.05). Plasma HDL cholesterol fraction was higher in rats fed 15% oils (p<0.05) and decreased with time of feeding (p<0.05). Triglycerides and VLDL concentrations were unaltered by all variables under study. The variations in LDL concentration originated from changes in the other lipid fractions measured, and exhibited a dependence on oil concentration and time of feeding.

TABLE 5  
PLASMA LIPID CONCENTRATIONS IN RATS FED THE EXPERIMENTAL DIETS FOR 15 OR 60 DAYS<sup>1</sup>

Dietary fat Type	Time %	Days	Choles-terol mmol/L	HDL mmol/L	LDL mmol/L	VLDL mmol/L	Triglyce-rides mmol/L
Corn oil	5	15	1.68±0.21	1.23±0.20	0.38±0.05	0.11±0.05	0.54±0.24
		60	1.31±0.35	0.73±0.20	0.30±0.18	0.16±0.05	0.79±0.26
	15	15	2.03±0.27	1.44±0.16	0.34±0.10	0.24±0.13	0.82±0.16
		60	2.02±0.22	1.12±0.18	0.71±0.09	0.19±0.06	0.93±0.29
Rose hip oil	5	15	1.24±0.35	1.09±0.19	0.25±0.05	0.17±0.04	0.76±0.28
		60	1.25±0.32	0.85±0.26	0.24±0.04	0.19±0.04	0.95±0.19
	15	15	1.87±0.12	1.38±0.19	0.25±0.14	0.25±0.14	1.25±0.68
		60	1.95±0.15	1.16±0.12	0.64±0.05	1.15±0.04	0.73±0.19

<sup>1</sup> Values are mean±S.D. (n=6). Significant effects of variables were evaluated with two-way analysis of variance (ANOVA). Significance levels are indicated under columns as follows:

Type of oil :	NS	NS	NS	NS	NS
Concentration :	p<0.05	p<0.05	p<0.05	NS	NS
Days of intake:	NS	p<0.05	p<0.05	NS	NS

## DISCUSSION

Highly polyunsaturated dietary fat intake produces changes in the membrane fatty acid composition of many tissues. The increase in PUFA content in membranes has been implicated in enhanced activity of enzymes such as Na, K-ATPase, lipoprotein lipase and ACAT(9,10), associated with increased fluidity. Na, K-ATPase, plays a crucial role in exchange processes with blood, affecting bile flow (11,12). The intake

of polyunsaturated oils may be expected to increase bile flow, due to their effect on enzyme activity. However, a direct relationship between membrane fluidity changes and bile flow is not always observed (13,14). None of the experimental conditions induced bile flow modifications in this study. Although dietary modulation of the fatty acid composition may have modified plasma membranes fluidity, the functional implication of this change did not affect bile flow. These results are in agreement with previous studies in which we (4) observed that the intake of high amounts of polyunsaturated oils did not alter the effect of ethinylestradiol, a cholestatic agent that decreases membrane fluidity (15,16).

Our results show that either vegetable oils exerted a significant effect on the biliary concentration of cholesterol or bile acids. Since the biliary concentration of PL was higher in rats fed RHO, it is possible that the intake of high amounts of PUFA, mainly of the (n-3) species, contributed to the synthesis of PL directed for biliary secretion. On the other hand, biliary lipids were not affected by dietary fat level, although C/PL ratio was higher in rats fed 15% oil diets, which also exhibited an increased molar percent cholesterol content. The most outstanding effect on bile lipids concerned the variable time. It exerted a clear-cut influence on the concentration of every biliary lipid species assayed, decreasing in all cases from 15 to 60 days of treatment.

When considering bile flow and lipid concentration, biliary lipid outputs showed similar dependence upon the variables studied. The increase in the time of feeding decreased bile lipid outputs in all groups. These results indicate that biliary lipid output is not dependent on the fatty acyl composition of ingested fats or their amount in the diet, but mainly on the length of feeding.

Dietary supplementation with PUFA is associated with a reduction in plasma cholesterol and triglyceride level (1). The hypocholesterolemic effect of unsaturated fats has been related to their ability to cause a redistribution of cholesterol between plasma and tissues (17). PUFA enhance hepatic LDL receptor activity (18), lipoprotein lipase and ACAT, increasing the cholesterol-ester content in the liver (9). The effect of PUFA is mediated by a decrease in liver lipid synthesis, depressing the formation of TG and VLDL (19). It is currently accepted that the exogenous supply of fatty acids affects the hepatic secretion of VLDL, an effect that is dependent upon their hydrocarbon chain length and saturation characteristics (20).

On a unit weight basis, (n-3) fatty acids have a greater hypocholesterolemic effect than the (n-6) fatty acids (21). Furthermore, the hypotriglyceridemic effect seems to be unique to (n-3) species, which are potent inhibitors of hepatic lipid synthesis (22,23). Balasubramaniam et al (24) observed a significant increase in biliary output of cholesterol in rats fed fish oil, rich in (n-3) fatty acids, concomitantly with a fall in plasma cholesterol levels. These authors proposed an alteration in hepatic plasma membranes to be responsible for an increased uptake of lipoproteins by the liver.

These or other mechanisms were expected to lower plasma lipid concentrations in RHO fed rats; however, our data reveal that the different fatty acid composition of CO and RHO had no effect on any of the lipid species determined, although an effect of dietary fat level was observed.

In summary, we have examined effects of two different vegetable oils on the composition of biliary and plasma lipids and found that the dietary fat level affected the plasma concentration of lipid. It may be concluded that, in spite of the greater P/S index of RHO, included its high amount of linolenic acid, both rose hip and corn oils exerted similar effects on the lipids measured. Together these results indicate that fat concentration and time of intake are the most important variables that determine the effects of dietary lipids on rat tissues.

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