Serum and Liver Lipids in Rats and Chicks Fed With Diets Containing Different Oils

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OBJECTIVES: Because dietary fat composition is determinant for serum cholesterol level, which is related to cardiovascular disease, we evaluated the effects of diets containing saturated (coconut oil) or polyunsaturated fatty acids (soybean oil) supplemented or not with dietary cholesterol on serum and liver lipid composition in two animal species.

METHODS: Male Wistar rats (21 d old) were assigned to one of seven groups and fed with commercial diet or diets containing 5% or 20% soybean oil or 20% coconut oil with or without 1% cholesterol. Chicks were assigned to one of four groups and fed with diets containing 15% soybean oil or 15% coconut oil with or without 1% cholesterol.

RESULTS: In rats, the accumulations of hepatic cholesterol and triacylglycerols were higher in the group fed 20% soybean oil and 1% cholesterol than in the group fed 20% coconut fat and 1% cholesterol. The highest serum levels of cholesterol and triacylglycerols were observed in the group fed coconut oil and cholesterol, compared with the group fed soybean oil and cholesterol. Triacylglycerol, high-density lipoprotein, and total cholesterol serum levels increased with diet containing coconut oil and cholesterol. In chicks, the highest hepatic cholesterol accumulation occurred in the group fed 15% coconut fat and 1% cholesterol. Total and high-density lipoprotein cholesterol levels increased with diet containing coconut oil and cholesterol, although none of these diets modified serum triacylglycerol levels.

CONCLUSIONS: The type of experimental animal model and the diet composition influence lipid metabolism. *Nutrition* 2003;19:789–793. ©Elsevier Inc. 2003

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INTRODUCTION

High dietary fat intake is a crucial risk factor for hypercholesterolemia, atherosclerosis, cardiovascular disease**,** and obesity. Dietary fat can affect the absorption of cholesterol, the hepatic cholesterol synthesis, the synthesis of biliary acids, and the number and activity of low-density lipoprotein (LDL) receptors.[1,2] Evidence shows that not only the amount of fat consumed but also the type of fatty acid influence serum cholesterol level.[3]

Recent studies have demonstrated that ingestion of polyunsaturated fatty acids (ω-3 and ω-6), present in vegetable oils, is inversely related to the incidence of heart disease by decreasing cholesterol and triacylglycerol plasmatic levels.[4,5] The main effect of ω-6 fatty acids is the reduction of cholesterol levels, mainly LDL. Fish oil, rich in ω-3 fatty acids, causes a substantial decline in very LDL (VLDL) concentrations and shows less effect on LDL levels. The effect of ω-3 fatty acids on the release of VLDL may be due to reduced levels of apoprotein B and microsomal triacylglycerol transfer protein. This reduction may lead to an increase in cholesterol and triacylglycerol levels in the liver and decrease their levels in serum.[6–8]

The present study investigated the effects of dietary fat and cholesterol on serum and liver lipid composition in two animal species (rats and chicks) by examining the influence of diets containing saturated fatty acids, mainly lauric and myristic (coconut oil), or polyunsaturated fatty acids**,** mainly linoleic (soybean oil). The effect of supplementation with cholesterol in both diets also was investigated.

MATERIALS AND METHODS

Materials

All chemicals used were of analytical reagent grade and purchased from Merck SA (Porto Alegre, Brazil).

Animals

Male Wistar rats (age, 21 d; weight range, 30–39 g) were obtained from a local breeding colony (Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul). They were maintained on a 12-h light-and-dark cycle in a ventilated room at 21°C with free access to food and water. One-day-old male chicks *(Gallus domesticus*; weight range, 11–13 g) were obtained from a commercial hatchery and maintained in a chamber with controlled temperature (28°C) with free access to food and water.

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TABLE I.

COMPOSITION OF THE COMMERCIAL DIET*

Composition	g/100 g diet
Total fat	11
Protein	22
Fiber	3
Ash	6
Vitamin	2
Carbohydrates	56

* Commercial non-purified diet from Nuvilab-CR1 (Curitiba, Brazil; caloric density, 4.16 cal/g).

The experimental protocol was developed according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, School of Veterinary Medicine and Animal Science of the University of São Paulo, Brazil.

Diets

Rats were randomly assigned to seven experimental groups, and each group received one of the following diets: regular laboratory chow (Nuvilab-CR1, Nuvital, Curitiba, Brazil), chow (g/100 g) plus 20% soybean oil (20% SO), chow plus 20% soybean oil and 1% cholesterol (20% SO + CHO), chow plus 20% coconut oil (20% CO), chow plus 20% coconut oil and 1% cholesterol (20% CO + CHO), chow plus 5% soybean oil (5% SO), or chow plus 5% soybean oil and 1% cholesterol (5% SO + CHO; Tables I and II). Food consumption was measured every 3 d, and weight gain was measured every week.

Chicks were fed with a commercial diet for 6 d. At age 1 wk, all chicks were weighed and randomly assigned to four experimental groups that received the diet plus 15% soybean oil (15% SO), diet plus 15% soybean oil and 1% cholesterol (15% SO + CHO), diet plus 15% coconut fat (15% CO), or diet plus 15% coconut oil and 1% cholesterol (15% CO + CHO; Table III). The percentile of fatty acid composition in each diet is listed in Table IV. All animals had free access to water and food immediately after hatching. Food consumption and weight gain were measured every week.

After treatment (6 wk for rats and 2 wk for chicks), all animals were killed by decapitation; blood samples were collected in test tubes and immediately centrifuged at 800*g* for 10 min to obtain serum samples. Livers were rapidly removed, weighed, and rinsed with 0.9% NaCl. Liver pieces and serum aliquots were stored at −70°C until biochemical analysis.

Serum Analysis

Biochemical analysis was performed in a Multi-test Analyzer (Mega, Merck, Darmstadt, Germany) by using specific kits supplied by Merck, as follows: triacylglycerols (SMT triacylglycerols, 1.19706.0001, with the GPO-PAP method), cholesterol (cholesterol SMT, 1.19738.0001, with the CHOD-PAP method), and LDL cholesterol (1.14992.0001, with the CHOD-PAP method). High-density lipoprotein (HDL) cholesterol was determined by using a kit (HDL cholesterol direct FS) from DiaSys (Diagnostic Systems International, Holzheim, Germany).

Liver Analysis

We measured levels of triacylglycerols, phospholipids, and cholesterol. Liver triacylglycerols were determined by the method of Soloni.[9] Liver lipids were extracted by the method of Folch et al.,[10] and phospholipid concentration was measured as the phosphate content of the lipid fraction according to the method of Fiske and Subbarow[11] after solvent evaporation and digestion of the lipid fraction with sulfuric acid. Liver cholesterol was determined by the method of Bergmeyer.[12]

Statistical Analysis

Data were analyzed by analysis of variance followed by Duncan's multiple-range test whenever values were initially significant. Sig-

TABLE II.

COMPOSITION OF THE EXPERIMENTAL DIETS GIVEN TO RATS*

Component	20% SO	20% SO + CHO	20% CO	20% CO + CHO	5% SO	5% SO + CHO
SO	20	20	—	—	5	5
CO	—	—	20	20	—	—
CHO	—	1	—	1	—	1
Soybean protein†	25	25	25	25	25	25
Sucrose	24	24	24	24	31	31
Cornstarch	24	24	24	24	31	31
Nonnutritive fiber	1	1	1	1	1	1
Salt mix‡	4	4	4	4	4	4
Vitamin mix§	1.5	1.5	1.5	1.5	1.5	1.5
L-methionine‖	0.15	0.15	0.15	0.15	0.15	0.15

* Caloric density: diets with 20% lipid, 4.72 cal/g; diets with 5% lipid, 3.99 cal/g.

† Samprosoy (Bunge Alimentos, Porto Alegre, Brazil).

‡ Mineral mixture (mg/100 g diet): NaCl, 557; Kl, 3.2; KH_2PO_4, 1556; $MgSO_4$, 229; $CaCO_3$, 1526; $FeSO_4 \cdot 7H_2O$, 108; $MnSO_4 \cdot H_2O$, 16; $ZnSO_4 \cdot 7H_2O$, 2.2; $CuSO_4 \cdot 5H_2O$, 1.9; and $CoCl_2 \cdot 6H_2O$, 0.09.

§ Vitamin mixture (Roche, São Paulo, Brazil; mg/100 g diet): vitamin A, 4; vitamin D, 0.5; vitamin E, 10; menadione, 0.5; choline, 200; PABA, 10; inositol 10; niacin, 4; panthothenic acid, 4; riboflavin, 0.8; thiamin, 0.5; pyridoxine, 0.5; folic acid, 0.2; biotin, 0.04; and vitamin B12, 0.003.

‖ Merck (Rio de Janeiro, Brazil).

CHO, cholesterol; CO, coconut oil; SO, soybean oil

TABLE III.

COMPOSITION OF THE EXPERIMENTAL DIETS GIVEN TO CHICKS*

Component	15% SO	15% SO + CHO	15% CO	15% CO + CHO
Soybean meal	35	35	35	35
Rice (polished and broken)	25	25	25	25
Wheat bran	20	20	20	20
Dicalcium phosphate	1.62	1.62	1.62	1.62
Limestone (calcium carbonate)	1.37	1.37	1.37	1.37
Common salt	0.49	0.49	0.49	0.49
Corn starch	1	—	1	—
Mineral Premix†	0.10	0.10	0.10	0.10
Antibiotic additive‡	0.06	0.06	0.06	0.06
Vitamin Premix§	0.05	0.05	0.05	0.05
DL-methionine‖	0.29	0.29	0.29	0.29
Antioxidant	0.02	0.02	0.02	0.02
CHO	—	1	—	1
SO	15	15	—	—
CO	—	—	15	15

* Caloric density: 3.10 cal/g.
† Mineral Premix supplied (per mg/kg): Mn, 60; Fe, 50; Zn, 40; Cu, 8; I, 0.4; Se, 0.3.
‡ Salinomycin 12% (for prevention of coccidiosis).
§ Vitamin Premix supplied (per kg of diet) 7000 IU of vitamin A, 1500 IU of vitamin D3, 30 mg of vitamin E, 2 mg of thiamin, 5 mg of riboflavin, 4 mg of piridoxine, 14 mg of pantothenic acid, 0.02 mg of vitamin B12 0.2 mg of biotin, 35 mg of niacin, 1 mg of folic acid, and 2 mg of vitamin K.
‖ From Degussa-Huels, Hanau, Germany.
CHO, cholesterol; CO, coconut oil; SO, soybean oil

TABLE IV.

PERCENTILE OF FATTY ACID COMPOSITION OF THE DIETARY FAT

Fatty acid	Soybean oil	Coconut oil
C8:0	0.0	2.4
C10:0	0.0	2.7
C12:0	0.0	44.4
C14:0	0.2	16.6
C16:0	10.6	9.6
C16:1 (ω-7)	0.4	0.0
C18:0	4.1	2.8
C18:1 (ω-9)	22.1	17.8
C18:2 (ω-6)	51.5	3.1
C18:3 (ω-3)	8.1	0.0
C20:0	0.4	0.1
C20:1	1.3	0.1
C22:0	0.5	0.0
C22:1	0.0	0.0
C24:0	0.2	0.0
Others	0.5	0.1

nificance level was set at $P < 0.05$. All analyses were done with SPSS 7.5 (SPSS, Chicago, IL, USA).

RESULTS

Rats

BODY WEIGHT. There was no statistically significant difference in body weight or weight gain between groups fed with 20% SO and groups fed with 20% CO. Rats on the 20% SO diet had greater weight gains than did those on the 5% SO diet. Food consumption was similar across groups (data not shown).

LIVER WEIGHT. Rats fed the CO-supplemented diets had heavier livers than did those fed the diets without cholesterol (mean ± standard error of the mean: without CO, 8.49 ± 0.42 g; with CO, 10.66 ± 0.33 g; $P \leq 0.05$).

LIVER AND SERUM LIPIDS. The effect of different types of dietary intake on hepatic and serum cholesterol, triacylglycerol, and phospholipid concentrations in rats is presented in Table V. The 20% SO + 1% CHO diet caused a higher hepatic cholesterol and triacylglycerol accumulation when compared with the 20% SO, 20% CO, and 20% CO + 1% CHO diets. The concentration of hepatic phospholipids was not modified by any diet. The influence of the different quantities of lipid in the diets on hepatic cholesterol and triacylglycerol accumulation is shown in Table V. The 20% SO + 1% CHO diet caused a four times higher hepatic cholesterol accumulation and a five times higher hepatic triacylglycerol accumulation when compared with the 5% SO + 1% CHO diet. We observed hypercholesterolemia and hypertriglyceridemia in rats fed the 20% CO diet. The greatest increase occurred with the 20% CO + 1% CHO diet. A significant reduction in HDL cholesterol levels occurred after feeding with the 20% SO + 1% CHO diet. After comparing the 20% SO and 5% SO diets with and without 1% CHO with the regular laboratory chow, we found that serum cholesterol level increased significantly with the 20% SO diets with and without 1% CHO in relation to the other three diets.

Chicks

BODY AND LIVER WEIGHT. These parameters were not affected by the experimental diets. Food consumption was similar across groups (data not shown).

LIVER AND SERUM LIPIDS. The feeding effect of different sources of fat on hepatic and serum cholesterol, triacylglycerols, and phospholipids in chicks is shown in Table VI. The hepatic cholesterol concentration was significantly higher in chicks fed with the CHO-supplemented diets. However, the most significant increase occurred with the 15% CO + 1% CHO diet. Liver triacylglycerol levels in the CO groups were significantly higher than those of the SO groups. The diets did not affect the levels of hepatic phospholipids. Total and HDL cholesterol levels increased with the 15% CO + 1% CHO diet. None of the diets affected serum triacylglycerol levels (Table VI).

DISCUSSION

Most studies related to lipid metabolism, usually with respect to ω-6 and ω-3 fatty acids, have used canola, sunflower, corn, and fish oils.[2,13,14] Soybean consumption has been increasing throughout the world, thus reinforcing the need for more research in this area. Soybean oil is one of the few vegetable oils that contain ω-3 and it is a significant source of ω-6 fatty acids.

Soybean or coconut oil was used as the single source for lipids in the diet offered to experimental animals. Soybean oil contains 53.7% of ω-6 fatty acid and 7.6% of ω-3 fatty acid,[3,15,16] and coconut oil contains 47.3% of lauric fatty acid and 18.2% of myristic fatty acid.[17] Our experiments compared not only the effect of different fatty acids in serum and hepatic lipid concentrations

TABLE V.

SERUM AND LIVER LIPIDS OF RATS FED WITH SOYBEAN OIL OR COCONUT OIL FOR 6 WK*

	Serum (mM/L)			Liver (μM/g)		
Group	CHO	HDL-C	Triacylglycerol	CHO	Triacylglycerol	Phospholipids
20% SO	2.81 ± 0.17	1.61 ± 0.08	1.55 ± 0.09	4.68 ± 0.52	5.01 ± 0.68	38.80 ± 1.20
20% SO + 1% CHO	3.04 ± 0.11	1.22 ± 0.03	1.47 ± 0.16	36.66 ± 2.60	26.33 ± 2.50*	40.10 ± 1.30
20% CO	3.56 ± 0.10	1.81 ± 0.10	2.12 ± 0.19	3.90 ± 0.52	4.78 ± 0.45	42.80 ± 1.10
20% CO + 1% CHO	4.08 ± 0.20	1.59 ± 0.05	2.84 ± 0.25	21.84 ± 2.60	15.04 ± 1.93	42.30 ± 1.00
5% SO	2.02 ± 0.04*	1.36 ± 0.03*	1.08 ± 0.09*	4.16 ± 0.26	1.93 ± 0.34	41.20 ± 1.10
5% SO + 1% CHO	2.03 ± 0.07*	1.17 ± 0.05	1.17 ± 0.13	10.40 ± 0.78	5.92 ± 1.25	40.00 ± 1.20
COM	1.64 ± 0.07*	0.98 ± 0.03	0.88 ± 0.05	5.20 ± 0.26	3.42 ± 0.45	36.80 ± 1.10

* Values are means ± standard error of the mean, $n = 8–16$. Different letters indicate statistical differences between oils by ANOVA with Duncan's post hoc test ($P < 0.05$). Different symbols indicate statistical differences between different SO quantities by ANOVA with Duncan's post hoc test ($P < 0.05$).

ANOVA, analysis of variance; CHO, cholesterol; CO, coconut oil; COM, commercial diet; HDL-C, high-density lipoprotein cholesterol; SO, soybean oil

but also the effect of lipid amount in the diet. Ros found that labeled cholesterol absorption from a fat-free meal is greatly reduced, indicating that the cholesterol absorption depends on the amount of fat in the diet.[1]

We found that the type of fatty acid influences the profile of hepatic lipid accumulation and that this influence depends on the animal species studied (Tables V and VI). In rats, the accumulation of hepatic cholesterol and triacylglycerols was higher in the group fed 20% SO + 1% CHO than in the group fed 20% CO + 1% CHO, whereas groups fed diets without the addition of cholesterol showed no differences (Table V). The hypolipidemic effect of ω-3 polyunsaturated fatty acid decreases the VLDL hepatic secretion that leads to an accumulation of triacylglycerols in the rat liver.[13,18] Gibbons et al. studied a diet rich in ω-3 fatty acids and suggested that the reduction in apoprotein B synthesis, mainly apoprotein B 48, has an important contribution to the reduction of VLDL synthesis.[19] Polyunsaturated fatty acid, when compared with the saturated fat in the experimental diets, induced a greater accumulation of hepatic cholesterol, mainly in the form of cholesterol ester, likely due to an increase in the ratio of acyl-coenzyme A to cholesterol acyltransferase activity by ω-6.[20] This fatty acid also increases the rate of bile acid synthesis and removal of chylomicron remnants from the blood.[18] The high concentration of cholesterol ester decreases free cholesterol concentration in the liver, thereby increasing LDL receptor synthesis and reducing serum cholesterol level. This action would explain the reduced cholesterol and triacylglycerol serum levels in the SO diets when compared with the diets containing saturated fat in our experiments (Table V).

In chicks, hepatic triacylglycerol accumulation was seven times higher in the CO group without cholesterol than in the SO group without cholesterol. This accumulation was 10 times higher in the CO + CHO group fed than in the SO + CHO group. As opposed to rats, hepatic cholesterol accumulation in chicks was greater with the 15% CO + 1% CHO diet than with the 15% SO + 1% CHO diet (Table VI).

Our results showed a higher concentration of serum cholesterol in chicks fed with saturated fat and cholesterol, which, according to the literature, is likely to be due to the slower uptake of chylomicron remnants originating from saturated fat.[21,22] A higher hepatic concentration of triacylglycerols (Table VI) and a higher hepatic synthesis of lipids from L-[U-^{14}C] alanine were observed in chicks fed with saturated fat (15% CO: 44.64 ± 4.11 pM/mg of liver per hour; 15% CO + 1% CHO: 50.0 ± 4.53 pM/mg of liver per hour; alanine converted to lipids) than in those fed with soybean oil (15% SO:13.41 ± 0.98 pM/mg of liver per hour; 15% SO + 1% CHO: 17.04 ± 0.78 pM/mg of liver per hour; alanine converted to lipids).

Some previous studies have shown that polyunsaturated fatty acid decreases the synthesis of fatty acid in the liver, increases β-oxidation, and reduces lipoprotein lipase activity of adipose tissue.[14,21] Lipoprotein hydrolysis, catalyzed by the lipoprotein

TABLE VI.

SERUM AND LIVER LIPIDS OF CHICKS FED SOYBEAN OIL AND COCONUT OIL FOR 2 WK*

	Serum (mM/L)			Liver (μM/g)		
Group	CHO	HDL-C	Triacylglycerol	CHO	Triacylglycerol	Phospholipids
15% SO	4.31 ± 0.21	2.23 ± 0.18	0.61 ± 0.02	5.98 ± 0.52	3.30 ± 0.34	39.90 ± 1.40
15% SO + 1% CHO	5.01 ± 0.26	2.51 ± 0.08	0.64 ± 0.06	11.44 ± 0.78	3.76 ± 0.34	41.50 ± 1.20
15% CO	5.20 ± 0.17	2.54 ± 0.02	0.63 ± 0.06	6.76 ± 0.52	20.74 ± 4.90	40.50 ± 1.60
15% CO + 1% CHO	6.68 ± 0.24	2.98 ± 0.08	0.68 ± 0.05	15.60 ± 1.30	37.16 ± 7.98	42.70 ± 2.10

* Values are means ± standard error of the mean, $n = 8–10$. Different letters indicate statistical differences across oils by analysis of variance with Duncan's post hoc test ($P < 0.05$).

HDL-C, high-density lipoprotein cholesterol; CHO, cholesterol; CO, coconut oil; SO, soybean oil

lipase, was higher with palmitic acid than with ω-6 and ω-3 fatty acids.[21,22] In the present study, in chicks, the level of serum triacylglycerols did not differ across the four groups. This result was likely due to hepatic lipid synthesis in groups fed saturated fat surpassing the lipoprotein lipase triacylglycerol hydrolysis, so that the accumulation in the liver becomes more evident than its effect in the blood. In contrast, the effect of the polyunsaturated fatty acids in the diet can be attributed to a reduction in the hepatic synthesis of fatty acids, which decreases the concentration of triacylglycerols in the liver and the blood.

Our results showed that, compared with a diet with 5% lipid, a hypercaloric diet (20% lipid) increases lipid concentrations in liver and serum. Chronically, this diet can induce cellular lipotoxicity. Indeed, one study found that diets with high lipid concentrations cause lipotoxicity in skeletal and heart muscles and in β-cells of Langerhans islets.[23] McGarry found that intramyocellular triacylglycerol concentrations have a higher positive correlation with insulin resistance than with any other parameter.[24,25]

In summary, this study demonstrated the distinct effects of saturated and polyunsaturated (coconut fat and soybean oil) fats and the additional effect of cholesterol supplementation in diets on serum and hepatic lipid levels. In addition, we showed an animal specificity in lipid metabolism, i.e., there were different effects of the studied diets on the analyzed parameters in rats and chicks.

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