Changes induced by cholesterol and fatty acids on the perinatal evolution of chick HMG-CoA reductase

Cambios inducidos por el colesterol y los ácidos grasos en la evolución perinatal de la HMG-CoA reductasa de pollo

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ABSTRACT

The effect of dietary fat quality on cholesterol metabolism in neonatal chick was examined in order to clarify the different mechanisms of hyper- and hypolipidemic action. Diet supplementation with 10% coconut oil produced a significant hypercholesterolemia after 7 days of treatment. Supplementation with 10% coconut oil plus 1% cholesterol produced a very higher increase of plasma cholesterol levels (about 2-3 times higher than those found with standard diet), while menhaden oil (10%) produced a significant decrease of total cholesterol. Hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase did not change by coconut oil treatment. However, both coconut oil plus cholesterol and menhaden oil supplemented diets drastically decreased reductase activity after 1 week of dietary manipulation. Our results suggest that different mechanisms may be implied in the regulation of cholesterol metabolism by the different fatty acids and/or cholesterol, especially at the level of reductase activity.

Key words: Cholesterol. HMG-CoA reductase. Coconut oil. Menhaden oil. Neonatal chick.

RESUMEN

Se ha estudiado el efecto de la calidad de la grasa dietaria sobre el metabolismo del colesterol, con objeto de verificar sus diferentes mecanismos de acción hiper- o hipocolesterolémica. La suplementación a la dieta con un 10% de aceite de coco produce una significativa hipercolesterolemia a los 7 días de tratamiento. La suplementación conjunta con un 10% de aceite de coco y un 1% de colesterol produce un incremento mucho mayor en los niveles de colesterol plasmático (alrededor de 2-3 veces superior al encontrado con la dieta estándar), mientras que el aceite de arenque (10%) produce un descenso significativo del colesterol total. La actividad HMG-CoA reductasa hepática no se modifica tras el tratamiento con aceite de coco. Sin embargo, las dietas suplementadas bien con aceite de coco más colesterol o bien con aceite de arenque disminuyen drásticamente la actividad reductasa después de 1 semana de manipulación

dietaria. Nuestros resultados sugieren que en la regulación del metabolismo del colesterol a nivel de la HMG-CoA reductasa por los diferentes ácidos grasos y/o colesterol pueden verse implicados distintos mecanismos de acción.
Palabras clave: Colesterol. HMG-CoA reductasa. Aceite de coco. Aceite de arenque. Pollo recién nacido.

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INTRODUCTION

Several epidemiological studies have shown a correlation between the consumption of different dietary components, the levels of blood lipids, mainly cholesterol, and the degree of atherosclerosis (1). Diets rich in saturated fat and/or cholesterol are associated with increased levels of plasma cholesterol and lipoprotein (2). Individuals of various animal species and human can differ in their susceptibility to dietary cholesterol (3) and other dietary constituents. The underlaying mechanisms of this variation are still poorly understood.

Recent data indicate that differences in the chain length of saturated fatty acids seem to influence plasma cholesterol concentration differently (4). Myristic acid (14:0) appears to be the principal saturated fatty acid that raises plasma cholesterol (5), increasing low-density lipoprotein (LDL) more than high-density lipoprotein (HDL). Differences in plasma LDL concentration could be accounted for by effects on LDL receptor-mediated catabolism and LDL flux rates.

On the other hand, it has been suggested that n-3 polyunsaturated fatty acids (PUFA) may protect against atherosclerotic vascular disease by different mechanisms including an increase in plasma lipid clearance and/or a decrease in lipid biosynthesis. However, the value on n-3 PUFA as agents for prevention or treatment of human atherosclerosis remains undetermined (6).

The chick has been recognized as a suitable animal model for studies on the comparative biochemistry of cholesterol metabolism and transport because it is highly sensitive to dietary cholesterol (7). After hatching, plasma cholesterol clearly decreases between 4 and 7 days, remaining practically constant from 7 days onwards (8). Likewise, cholesterol content in neonatal chick liver sharply decreases during the first days after hatching (9).

Because of these considerations, in this paper we have studied the influence of diet supplementation with saturated fatty acids (coconut oil), without or with added cholesterol, and of n-3 PUFA (menhaden oil) on the plasma and liver lipid composition along the neonatal development of chick. Bearing in mind the uncertainty regarding the effect of dietary fat quality on endogenous cholesterol synthesis and the postnatal changes in chick liver 3-hydroxy-3-

methylglutaryl coenzyme A (HMG-CoA) reductase (10), the main regulatory enzyme of cholesterogenesis, we have also studied the effect of the same dietary treatments on hepatic cholesterol synthesis by measurement of this microsomal enzyme activity.

MATERIALS AND METHODS

Newborn White Leghorn male chicks (*Gallus domesticus)* were obtained from a commercial hatchery and maintained in a chamber with a light cycle from 09.00 to 21.00 h and controlled temperature (28 °C). Newborn animals were randomly divided into three groups of 15 chicks each and were fed a standard diet supplemented with 10% saturated fat (coconut oil), with 10% saturated fat plus 1% cholesterol or with 10% polyunsaturated fat (menhaden oil). Another group of control animals were fed on a commercial diet (Sanders A-00). All animals had free access to water and food. The feeding was continued for 2 weeks.

The coconut oil was for pharmaceutical use (Acofarma). The menhaden oil was supplied by ICN Biochemicals, Cleveland, USA, and was stored under nitrogen at –20 °C. All other reagents were analytical grade. All diets were prepared daily to minimize the oxidation deterioration. The fatty acid compositions of different dietary regimens are shown in Table 1. No significant differences were observed in fatty acid composition of each diet during the experiments. Simultaneous supplementation of 1% cholesterol plus 10% coconut oil did not change significantly the fatty acid composition of this diet with respect to that of coconut oil diet.

After each treatment, blood was taken from each animal by decapitation after 12 h food deprivation and was kept at 4 °C for 2 h. Plasma was separated by centrifugation at 2500 rpm for 20 min at 4 °C. Livers were rapidly removed, minced and then homogenized with a motor-driven all glass Potter-Elvehjem homogenizer in 3 vol of 50 mM phosphate buffer, pH 7.4, containing 30 mM EDTA, 250 mM NaCl and 1 mM dithiothreitol. Microsomes were obtained as previously described (11). Plasma and tissue lipids were extracted with chloroform/ methanol 2:1 (v/v) as described by Folch et al. (12). Total and free cholesterol as well as triacylglycerol contents were determined by enzymic colorimetric methods by using "Test-Combination Cholesterol", "Test-Combination Free Cholesterol" or "GPO-PAP Test" respectively, from Boehringer Mannheim, Mannheim, Germany. Protein concentration was determined by the method of Lowry et al. (13) using bovine albumin as a standard. HMG-CoA reductase activity was measured essentially as described by Shapiro et al. (14). Other details of assay conditions have been reported elsewhere (10).

Table 1.—Fatty acid composition of diets

Fatty acid	*Control*	*CO*	*M*
8:0		1.7	
10:0		3.0	
12:0		29.0	0
14:0	0.8	12.4	6
16:0	22.3	14.9	22
18:0	8.6	5.6	5
Total Sat.	31.7	67.5	34
16:1 n-7	3.3	1.3	9
18:1 n-9	32.4	17.4	19
20:1 n-9			0
Total MUFA	35.7	18.7	29
18:3 n-3	0.8	0.3	0
20:5 n-3			12.0
22:5 n-3	1.7	0.7	1.6
22:6 n-3			6.8
Total n-3	2.5	1.0	21.0
18:2 n-6	24.6	10.8	10.7
20:2 n-6	2.5	1.0	2.4
20:3 n-6	1.1	0.5	0.6
20:4 n-6	1.6	0.6	1.4
Total n-6	29.8	12.9	15.1
Sat./Unsat	0.46	2.07	0.5
Sat./PUFA	0.98	4.85	0.9
n-3/n-6	0.08	0.08	1.3

CO, diet supplemented with 10% coconut oil; MO, diet supplemented with 10% menhaden oil.

Three experiments with pools of 6 animals were performed in each case. Triplicate determinations were carried out in each experiment. Student's *t* test was used for unpaired groups.

RESULTS

In spite of the different fat content of the different diets, chicks consumed similar amounts of food irrespective of the dietary regimen. No significant differences in body weight gain among groups fed different diets were observed. Therefore, supplementation of the diet with coconut oil (with or without cholesterol) or menhaden oil did not interfere with the growth rate of animals.

Supplementation of 10% coconut oil to the diet produced a significant hypercholesterolemia after 7 days of treatment (Table 2). Total cholesterol

levels in plasma was maintained practically constant from 7 days onwards. This hypercholesterolemia seems to be due to the increase in both free and esterified cholesterol, although differences induced in the percentage of both forms by coconut oil were not significant (Table 3): the esterified form represented more than 60% of total cholesterol. Simultaneous supplementation of 10% coconut oil and 1% cholesterol produced a sharp increase of plasma cholesterol levels, reaching values about 2-3 times higher than those obtained with standard diet. However, supplementation of 10% menhaden oil to the diet produced a significant decrease in plasma cholesterol levels when compared with control animals. This decrease was higher in the esterified than in the free cholesterol, so that the percentages of both forms tend to be practically similar after this treatment (Table 3).

Data in Table 2 show that 10% coconut oil did not affect significantly to the plasma triacylglycerol content, even after 2 weeks of treatment. However, simultaneous supplementation of 1% cholesterol to the coconut diet produced a significant increase in plasma triglycerides with respect to the control animals. Menhaden oil supplementation clearly decreased the levels of triacylglycerols in plasma after 1 week of treatment, although when dietary supplementation of menhaden oil was prolonged for 2 weeks these levels were similar to those found in control animals.

Table 2.—Effects of diet supplementation with 10% coconut oil (without or with 1% cholesterol) and 10% menhaden oil on lipid composition of neonatal chick plasma

	Component (mmol/L)		
	Total Cholesterol	*Free Cholesterol*	*Triglycerides*
1 week			
Control	4.19±0.10	1.58±0.23	0.48±0.04
CO	5.12±0.07^{b}	1.99±0.08	0.56±0.03
CO + CHO	13.67±0.03^{c3}	4.01±0.60^{a1}	0.86±0.12^{a}
MO	2.27±0.28^{b2§}	1.03±0.18^{1*}	0.17±0.07^{a2*}
2 weeks			
Control	4.39±0.15	1.06±0.18	0.55±0.03
CO	5.58±0.10^{b}	1.52±0.05	0.68±0.04
CO + CHO	13.56±2.29^{a1}	4.03±0.95^{a}	1.03±0.13^{a}
MO	2.20±0.34^{b2*}	0.93±0.10*	0.53±0.07*

Results are expressed as mean values±SEM of three experiments carried out with pools of 6 animals. Triplicate determinations were made in each experiment.

CO, diet supplemented with 10% coconut oil; CO + CHO, diet supplemented with 10% coconut oil + 1% cholesterol; MO, diet supplemented with 10% menhaden oil.

^{a,b,c} Significantly different from control: $^{a}P<0.05$; $^{b}P<0.005$; $^{c}P<0.0005$

^{1,2,3} Significantly different from CO: $^{1}P<0.05$; $^{2}P<0.005$; $^{3}P<0.0005$

*,†,§ Significantly different from CO + CHO: $^{*}P<0.05$; $^{\dagger}P<0.005$; $^{\S}P<0.0005$

Table 3.—Effects of diet supplementation with 10% coconut oil (without or with 1% cholesterol) and 10% menhaden oil on percentages of free and esterified cholesterol in neonatal chick

	% Free Cholesterol	*% Esterified Cholesterol*
1 week		
Control	37.65±5.02	62.35± 5.76
CO	38.89±1.62	61.11± 2.22
CO + CHO	29.30±8.12	70.70± 8.14
MO	45.45±9.77	54.55±16.27
2 weeks		
Control	24.12±4.20	75.88± 5.33
CO	27.31±1.05	72.69± 2.67
CO + CHO	29.71±8.66	70.29±21.82
MO	42.35±6.50	57.65±12.22

Results are expressed as mean values±SEM of three experiments carried out with pools of 6 animals. Triplicate determinations were made in each experiment.
CO, diet supplemented with 10% coconut oil; CO + CHO, diet supplemented with 10% coconut oil + 1% cholesterol; MO, diet supplemented with 10% menhaden oil.

The hypercholesterolemic effect of coconut oil was not accompanied by changes in the levels of liver cholesterol. Results in Table 4 demonstrate that hepatic cholesterol did not change significantly even after 2 weeks of 10% coconut oil treatment. Nevertheless, the simultaneous addition of 10% coconut oil plus 1% cholesterol to the diet produced a sharp increase in liver cholesterol. When this dietary treatment was prolonged for 2 weeks, differences were more patent in the levels of esterified cholesterol. At this age, cholesteryl esters content decreased in control animals while cholesterol supplementation to the diet drastically increased their percentage (Table 5).

Supplementation to the diet whith coconut oil plus cholesterol for 1 week induced a slight but significant decrease of hepatic triacylglycerol content (Table 4). However, when this treatment was prolonged for 2 weeks, triglycerides values seem to be similar to those found in control animals. No other of the dietary regimens assayed produced significant changes in the levels of triglycerides in chick liver.

Hepatic HMG-CoA reductase activity in neonatal chicks treated with coconut oil for 1-2 weeks did not change significantly with respect to the control values. A similar age-related increase was observed with or whithout coconut oil supplementation to the diet. However, in animals simultaneously treated with coconut oil plus cholesterol, microsomal HMG-CoA reductase activity was drastically reduced (Table 6). Menhaden oil treatment also induced a strong decrease in HMG-CoA reductase activity, especially after the first week of treatment.

Table 4.—Effects of diet supplementation with 10% coconut oil (without or with 1% cholesterol) and 10% menhaden oil on lipid composition of neonatal chick liver

	Component (imol/g tissue)		
	Total Cholesterol	*Free Cholesterol*	*Triglycerides*
1 week			
Control	19.22±1.96	10.44±1.39	7.62±0.68
CO	21.65±1.85	10.26±0.87	6.59±0.11
CO + CHO	34.83±1.57[b1]	16.85±1.06[a1]	4.24±0.14[a3]
MO	13.51±1.21[1§]	9.04±1.03[*]	5.31±0.80
2 weeks			
Control	8.63±1.03	7.42±0.54	6.66±1.05
CO	9.61±0.72	9.12±0.10[a]	5.16±0.64
CO + CHO	33.75±3.87[b2]	15.74±2.52[a]	4.90±0.47
MO	8.29±0.62	7.05±0.56[1*]	5.25±0.46

Results are expressed as mean values±SEM of three experiments carried out with pools of 6 animals. Triplicate determinations were made in each experiment.
CO, diet supplemented with 10% coconut oil; CO + CHO, diet supplemented with 10% coconut oil + 1% cholesterol; MO, diet supplemented with 10% menhaden oil.
[a,b,] Significantly different from control: [a]P<0.05; [b]P<0.005
[1,2,3] Significantly different from CO: [1]P<0.05; [2]P<0.005; [3]P<0.0005
[*,†,§] Significantly different from CO + CHO: [*]P<0.05;[†]P<0.005; [§]P<0.0005

Table 5.—Effects of diet supplementation with 10% coconut oil (without or with 1% cholesterol) and 10% menhaden oil on percentages of free and esterified cholesterol in neonatal chick liver

	% Free Cholesterol	*% Esterified Cholesterol*
1 week		
Control	56.58±9.66	47.62±13.97
CO	47.37±5.74	52.63±10.45
CO + CHO	48.37±6.32	51.63±7.77
MO	66.92±9.72	33.08±12.03
2 weeks		
Control	85.93±12.05	14.07±2.15
CO	94.89±7.22	5.11±0.84
CO + CHO	46.63±9.21[1]	53.37±15.01[1]
MO	85.05±9.34[*]	14.95±1.49

Results are expressed as mean values±SEM of three experiments carried out with pools of 6 animals. Triplicate determinations were made in each experiment.
CO, diet supplemented with 10% coconut oil; CO + CHO, diet supplemented with 10% coconut oil + 1% cholesterol; MO, diet supplemented with 10% menhaden oil.
[1] Significantly different from CO: [1]P<0.05
[*] Significantly different from CO + CHO: [*]P<0.05

Table 6.—Effects of diet supplementation with 10% coconut oil (without or with 1% cholesterol) and 10% menhaden oil on HMG-CoA reductase activity

	HMG-CoA reductase activity (pmol/min . mg protein)	
	1 wee	*2 weeks*
Control	422 ± 50	737 ± 99
CO	467 ± 30	741 ± 45
CO + CHO	263 ± 25[a1]	188 ± 18[a3]
MO	148 ± 31[a2*]	230 ± 41[a2]

Results are expressed as mean values±SEM of three experiments carried out with pools of 6 animals. Triplicate determinations were made in each experiment.
CO, diet supplemented with 10% coconut oil; CO + CHO, diet supplemented with 10% coconut oil + 1% cholesterol; MO, diet supplemented with 10% menhaden oil.
[a] Significantly different from control. [a]P<0.05
[1,2,3] Significantly different from CO: [1]P<0.05; [2]P<0.005; [3]P<0.0005
[*] Significantly different from CO + CHO: [*]P<0.05

DISCUSSION

Our results indicate an interactive influence of saturated fat and cholesterol when both constituents were supplemented simultaneously to the diet. Recent data from our laboratory indicate that neonatal chick, in which there are higher levels of plasma cholesterol than in adult chick, responds more slowly to 2% cholesterol supplementation to the diet: a significant hypercholesterolemia was observed only after 15 days of this treatment in newborn chicks, while a similar effect was found after 3 days of the same treatment in adult chicks (15). Our results show a strong increase of this responsivennes when cholesterol was administered simultaneously to coconut oil in the diet. These finding may be interpreted on the basis of the "cholesterol vehicle" function of these fats, which may augment intestinal absorption of cholesterol and, therefore, its hypercholesterolemic effect (16).

Likewise, coconut oil supplementation did not change the amounts and percentages of both free and esterified cholesterol in liver, while the simultaneous addition of cholesterol strongly increased the levels of esterified cholesterol, reaching a percentage of this form higher than 50%. These finding are in agreeement with those previously obtained by cholesterol supplementation to the standard diet (17).

Sterol balance studies have reported various responses in cholesterol synthesis from changes in dietary fat quality. The lack of response of neonatal chick liver HMG-CoA reductase to a coconut oil supplemented diet coincides with our previous results working with 14-day-old chicks: no significant difference was found in HMG-CoA reductase activity by supplementation to the diet with 10 or 20% coconut oil for 1-2 weeks (18).

On the other hand, the clear inhibition of HMG-CoA reductase activity found by coconut oil plus cholesterol feeding is in agreement with that previously reported in neonatal chicks when were fed a diet supplemented *with 2% cholesterol during the 2 first weeks after hatching (19).* The hypercholesterolemic effect of cholesterol was generally acompanied by an accumulation of cholesterol in liver, interfering with the hepatic cholesterogenesis. Thus, the clear increase found in hepatic cholesterol after coconut oil plus cholesterol feeding may be related with the strong inhibition of hepatic HMG-CoA reductase activity.

The mechanisms involved in the hypocholesterolemic effects of polyunsaturated fats have not been clearly defined. An increase in hepatic LDL receptors have been proposed (20). Recently, Mizuguchi et al. (21) have reported that a highly purified ethyl ester of all-cis-5,8,11,14,17-icosapentaenoate (EPA-E) significantly inhibited rat liver HMG-CoA reductase, while Fernandez et al. (22) have shown that guinea pigs fed different diets rich in 16:0, 18:1 or 18:2 n-6 fatty acids had similar values for hepatic HMG-CoA reductase activity, in spite of differences found in plasma total and lipoprotein cholesterol, suggesting that regulation of hepatic HMG-CoA reductase activity is relatively independent of changes in plasma lipoprotein levels.

It has been suggested that the decreased activity of microsomal HMG-CoA reductase caused by EPA-E may be due to the increase in microsomal free cholesterol (21). However, results not shown obtained in our laboratory seem to indicate that no significant differences were observed in free cholesterol from chick microsomes after supplementation of menhaden oil to the diet, in contrast to the rapid increase of cholesterol/phospholipid molar ratio found in chick microsomes after cholesterol feeding (23). Changes in fatty acid composition of microsomal phospholipids may be related with the inhibition of HMG-CoA reductase found after menhaden oil administration.

Putative mechanisms underlying the major metabolic effects of fish oil include an increase in plasma lipid clearance beside the decrease in lipid biosynthesis. More studies would be necessary to obtain conclusive information on the effects of fish oil on chick lipoprotein metabolism. However, data in this paper suggest that different mechanism(s) may be implied in the regulation of cholesterol metabolism by the different fatty acids and/or cholesterol at the level of HMG-CoA reductase activity.

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