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Differential effects of dietary fat on chick plasma and liver composition and HMG-CoA reductase activity

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The comparative effects of diet supplementation with 10% saturated fat rich in 12:0 and 14:0 fatty acids (coconut oil), without and with 1% added cholesterol, and with 10% unsaturated fat rich in n-3 polyunsaturated fatty acids (menhaden oil) on cholesterol metabolism in neonatal chicks were examined to clarify the different mechanisms of their hyper- and hypolipidemic action. Supplementation of coconut oil produced a significant hypercholesterolemia after 7 days of treatment, with a similar increase in the amount of both free and esterified cholesterol. Supplementation of coconut oil plus cholesterol produced a higher increase of plasma cholesterol levels (approximately two to three times higher than those found with standard diet). However, supplementation of menhaden oil induced a significant decrease in total cholesterol after only 2 weeks of treatment. Levels of plasma triglycerides did not change by coconut oil addition to the diet, but a significant increase was observed after coconut oil plus cholesterol feeding. Menhaden oil produced a transient decrease in plasma triglycerides. Hepatic 3-hydroxy-3-methylglutaryl-CoA reductase activity did not change with coconut oil treatment. However, both coconut oil plus cholesterol and menhaden oil supplemented diets drastically decreased reductase activity after 1 week of dietary manipulation. These results show that different nutrients with the same inhibitory effect on reductase activity produced opposite effects on plasma cholesterol content, suggesting the existence of important differences in the regulatory mechanisms implied in cholesterol biosynthesis and its accumulation in plasma. (J. Nutr. Biochem. 10:198–204, 1999)

Keywords: cholesterol; HMG-CoA reductase; coconut oil; menhaden oil; neonatal chick

Introduction

Several epidemiologic 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] A major point of interest has been the way dietary fatty acids and cholesterol affect these blood lipids. Diets rich in saturated fat and/or cholesterol are associated with increased levels of plasma cholesterol[2,3] and lipoprotein.[4] Despite the vast body of data, controversy persists concerning the effects of specific fatty acids, what effect dietary cholesterol has on this relationship, and the underlying mechanism of action.[5] Various animal species and humans can differ in their susceptibility to dietary cholesterol[6] and other dietary constituents. The underlying mechanisms of this variation are still poorly understood.

Recent data indicate that saturated fatty acids cannot be regarded as a single entity, because differences in the chain length seem to influence plasma cholesterol concentration differently.[7,8] Myristic acid (14:0) appears to be the principal saturated fatty acid that raises plasma cholesterol,[9] increasing low density lipoprotein (LDL) more than high density lipoprotein (HDL).[7] Differences in plasma LDL concentration could account for the effects on LDL recep-

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tor-mediated catabolism and LDL flux rates,[10,11] whereas differences in plasma HDL concentrations seem to be related to variations in apo A-I production rates.[12]

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 of n-3 PUFA as agents for prevention or treatment of human atherosclerosis remains undetermined.[13]

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.[14] Extensive changes in the lipid composition and metabolism of plasma, liver, and other tissues occur during embryonic development of the chick.[15] After hatching, plasma cholesterol clearly decreases between 4 and 7 days, remaining practically constant from 7 days on.[16] Likewise, cholesterol content in neonatal chick liver sharply decreases during the first days after hatching.[17] These rapid changes in cholesterol content in different tissues render the newborn chick an interesting model for the study of cholesterol metabolism throughout postnatal development.

However, we have found different hypercholesterolemic effects of cholesterol and saturated fat (coconut oil) on neonatal and adult chicks.[18,19] Our studies have shown that newborn chicks are more sensitive to saturated fatty acids with 12 and 14 carbons than adult animals and that supplementation of 0.5 to 2.0% cholesterol to the diet produced similar hypercholesterolemic effects. Our results show that the neonatal chick provides a suitable model by which to study the role of very low density lipoproteins (VLDL) in atherogenesis and the rapid response to saturated fatty acids with 12 and 14 carbons.[20] We also have showed a strong increase of this responsiveness when cholesterol was administered simultaneously with coconut oil in the chick diet,[21] which is in contrast to the different interactive influence observed in humans.[22,23] This discrepancy may be accounted for by the different susceptibility of different animal species to dietary cholesterol. Thus, it has been reported that certain differences exist among human and laboratory animals with respect to responsiveness of plasma lipid levels to changes in dietary cholesterol.[24,25] In this sense, it has been proposed that the complexity of the metabolic basis of diet responsiveness suggests the involvement of many genes in determining the response of phenotypes.[26] Our recent results on young chick also show a response to coconut oil, without interference of diet or tissue cholesterol, that is more rapid than that reported in humans and other animal species: A significant hypercholesterolemic effect was observed after 1 day of this dietary manipulation.[27]

Because of these considerations, we studied the comparative 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 during 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-CoA (HMG-CoA) reductase,[28] the main regulatory enzyme of cholesterogenesis, we also studied the effect of the same dietary treatments on hepatic cholesterol synthesis by measurement of this microsomal enzyme activity.

Table 1 Fatty acid composition of experimental diets (percentage of total fatty acids)

Fatty acid	Control	CO	MO
8:0		1.7	
10:0		3.0	
12:0		29.0	0.1
14:0	0.8	12.4	6.6
16:0	22.3	14.9	22.3
18:0	8.6	5.6	5.8
Total saturated	31.7	67.5	34.8
16:1 n-7	3.3	1.3	9.2
18:1 n-9	32.4	17.4	19.5
20:1 n-9			0.4
Total MUFA	35.7	18.7	29.1
18:3 n-3	0.8	0.3	0.6
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
Saturated/unsaturated	0.46	2.07	0.53
Saturated/PUFA	0.98	4.85	0.96
n-3/n-6	0.08	0.08	1.39

CO–standard diet supplemented with 10% (w/w) coconut oil. MO–standard diet supplemented with 10% (w/w) menhaden oil. MUFA–monounsaturated fatty acids. PUFA–polyunsaturated fatty acids.

Materials and methods

Newborn white leghorn male chicks (*Gallus domesticus*) were obtained from a commercial hatchery (Granja Avícola Santa Isabel, Córdoba, Spain) and maintained in a chamber with a light cycle from 9:00 AM to 9:00 PM and controlled temperature (28°C). Newborn animals were randomly divided into three groups of 15 chicks each and were fed the standard diet supplemented with 10% (w/w) saturated fat (coconut oil), with 10% (w/w) saturated fat plus 1% (w/w) crystalline free cholesterol, or with 10% (w/w) polyunsaturated fat (menhaden oil). All additives were mixed homogeneously with the standard diet. Another group of control animals were fed on the standard diet (Sanders A-00, Granada, Spain), which contained (w/w) 45.2% carbohydrate (mainly starch), 6.6% fat, and 20.5 protein. This standard diet is normally used for growing chick. All animals had free access to water and food. The feeding was continued for 2 weeks. As previously reported, this feeding period is sufficient to achieve the studied response.

The coconut oil was for pharmaceutical use (Acofarma, Spain). The menhaden oil was supplied by ICN Biochemicals (Cleveland, OH USA) and was stored under nitrogen at −20°C. Radioactive reagents [3-^{14}C]HMG-CoA and [2-^{3}H]mevalonic acid lactone (MVA) were supplied by Amersham International (Amersham, Little Chalfont, Buckinghamshire, UK). All other reagents were of analytical grade. All diets were prepared daily to minimize the oxidation deterioration. Fatty acid composition of analyzed diets is given 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 significantly change 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 hours of food deprivation and was kept at 4°C for 2 hours. Plasma was separated by centrifugation at 2,500 rpm for 20 minutes at 4°C. Livers were rapidly removed, minced and homogenized with a motor-driven all-glass Potter-Elvehjem homogenizer in 3 volumes of 50 mM phosphate buffer, pH 7.4, containing 30 mM ethylenediamine tetraacetic acid (EDTA), 250 mM NaCl, and 1 mM dithiothreitol. Microsomes were obtained as previously described.[29] Plasma and tissue lipids were extracted with chloroform/methanol 2:1 (v/v) as described by Folch et al.[30] Total and free cholesterol, as well as triacylglycerol contents, were determined by enzymatic colorimetric methods by using Test-Combination Cholesterol, Test-Combination Free Cholesterol, or GPO-PAP Test, respectively, obtained from Boehringer Mannheim GmbH (Mannheim, Germany). Protein concentration was determined by the method of Lowry et al.[31] using bovine albumin as a standard. HMG-CoA reductase activity was measured essentially as described by Shapiro et al.[32] This method measured the formation of [^{14}C]MVA from [^{14}C]HMG-CoA, using [^{3}H]MVA as an internal standard. Other details of assay conditions have been reported elsewhere.[28]

Three experiments with pools of six animals were performed in each case. Triplicate determinations were carried out in each experiment. Data were analyzed by two-way analysis of variance (ANOVA). When overall F-statistic was significant ($P < 0.05$), analyses of significance were made by the Student's *t*-test.

Results

In spite of the different fat content of the different diets, chicks consumed similar amounts of foods, irrespective of the dietary regimen. No significant differences in body and liver weight gain were observed among groups fed different diets. 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 were maintained practically constant from 7 days on. This hypercholesterolemia seems to be due to the increase in both free and esterified cholesterols, 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 approximately two to three times higher than those obtained with standard diet. However, supplementation of 10% menhaden oil to the diet produced a slight decrease in plasma cholesterol levels when compared with control animals. This decrease was significant only after 2 weeks of treatment and was higher in the esterified than in the free cholesterol, so that percentages of both forms tend to be practically similar after this treatment (*Table 3*).

Data in *Table 2* also show that 10% coconut oil did not affect 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.

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

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

Diets	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	3.38 ± 0.28^{1†}	1.53 ± 0.18*	0.30 ± 0.03^{a2*}
2 weeks			
Control	4.44 ± 0.16	1.07 ± 0.18	0.55 ± 0.03
CO	5.58 ± 0.10^{a}	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	2.79 ± 0.52^{a1*}	1.18 ± 0.10*	0.53 ± 0.07*

Note: Results are expressed as mean values ± SEM of three experiments carried out with pools of six animals. Triplicate determinations were made in each experiment: CO–standard diet supplemented with 10% (w/w) coconut oil. CO + CHO–standard diet supplemented with 10% (w/w) coconut oil + 1% (w/w) cholesterol. MO–standard diet supplemented with 10% (w/w) menhaden oil.
Significantly different from control: $^{a}P < 0.05$; $^{b}P < 0.005$; $^{c}P < 0.0005$.
Significantly different from CO: $^{1}P < 0.05$; $^{2}P < 0.005$; $^{3}P < 0.0005$.
Significantly different from CO + CHO: $^{\star}P$ 0.05; †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 plasma

Diets	% 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

Note: Results are expressed as mean values ± SEM of three experiments carried out with pools of six animals. Triplicate determinations were made in each experiment: CO–standard diet supplemented with 10% (w/w) coconut oil. CO + CHO–standard diet supplemented with 10% (w/w) coconut oil + 1% (w/w) cholesterol. MO–standard diet supplemented with 10% (w/w) menhaden oil.

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

Diets	Component (μmol/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

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

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 whereas cholesterol supplementation to the diet drastically increased their percentage (*Table 5*).

Supplementation of coconut oil plus cholesterol to the diet for 1 week induced a slight but significant decrease of hepatic triacylglycerol content (*Table 4*). However, when this treatment was prolonged for 2 weeks, triglyceride

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

Diets	% 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

Note: Results are expressed as mean values ± SEM of three experiments carried out with pools of six animals. Triplicate determinations were made in each experiment: CO–standard diet supplemented with 10% (w/w) coconut oil. CO + CHO–standard diet supplemented with 10% (w/w) coconut oil + 1% (w/w) cholesterol. MO–standard diet supplemented with 10% (w/w) menhaden oil.
[1]Significantly different from CO: $P < 0.05$.
*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

Diets	HMG-CoA reductase activity (pmol/min/mg protein)	
	1 week	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]

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

values seem to be similar to those found in control animals. None of the other dietary regimens assayed produced significant changes in the levels of triglycerides in chick liver.

Hepatic HMG-CoA reductase activity from neonatal chicks fed coconut oil diet for 1 to 2 weeks did not change significantly with respect to the control values. A similar age-related increase was observed with or without coconut oil supplementation to the diet. However, in animals simultaneously treated with coconut oil plus cholesterol, microsomal HMG-CoA reductase activity was reduced drastically (*Table 6*). Menhaden oil also induced a strong decrease in HMG-CoA reductase activity, especially after the first week of treatment.

Discussion

Although the effects of dietary fat and cholesterol on circulating cholesterol levels have been well defined, changes that occur in cholesterol metabolism to account for these dietary effects are less clear. Furthermore, independent and interactive mechanisms of action of these dietary constituents have not been fully defined.[23] Recent data from our laboratory indicate that the 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: Significant hypercholesterolemia was observed only after 15 days of this treatment in newborn chick, whereas a similar effect was found after 3 days of the same treatment in adult chick.[19] Our current results indicate a synergic influence of saturated fat and cholesterol when both constituents were supplemented simultaneously in the diet. These findings 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.[33]

It has been proposed that animals with high responses have a higher efficiency of cholesterol absorption. In agreement with this reasoning is one study that found that cholesterol feeding elevated liver cholesterol concentrations

to a greater extent in hyperresponsive than in hyporesponsive animals.[34] Our results show that coconut oil plus cholesterol feeding drastically increased total cholesterol in chick liver. This increase was particularly patent after 2 weeks of dietary manipulation (14-day-old chicks), mainly due to the low levels observed at this age in control animals as a result of the changes occurring during postnatal development.[35]

Measurements of hepatic HMG-CoA reductase activity also resulted in conflicting reports on the effects of dietary fat quality. Two major variables in many studies are the amount of dietary fat fed and the choice of saturated fat used, whether coconut oil, with a high content of short-chain saturated fatty acids, or a saturated fat containing predominantly palmitic and stearic acids.[36] Recent data from Fernandez et al.[37] indicate that guinea pigs fed a palm kernel diet containing more than 70% of 12:0 and 14:0 fatty acids had lower HMG-CoA reductase activity compared with other dietary groups, especially beef tallow diet, which contained more than 70% of 16:0 and 18:1 fatty acids. Our previous results working with 14-day-old chicks showed that no significant difference was found in HMG-CoA reductase activity by supplementation to the diet with 10% or 20% coconut oil for 1 to 2 weeks when animals also were euthanized after fasting.[18] The lack of saturated fat effects observed in our experiments is not attributable to food restriction. Although we have found that levels of HMG-CoA reductase activity were lower in fasted animals than in normal fed chicks,[38] more recent data confirmed that levels of this enzyme activity were higher when chicks were euthanized 6 hours after free access to food than those euthanized after a 12-hour fast, but no changes were observed after coconut oil feeding for 1 to 2 weeks.[39]

On the other hand, the clear inhibition of HMG-CoA reductase activity induced by coconut oil plus cholesterol feeding is in agreement with that previously observed in neonatal chicks that were fed a diet supplemented with 2% cholesterol during the 2 first weeks after hatching.[40] This inhibition was found when animals were euthanized approximately 12 hours after food deprivation, as in this work. Levels of HMG-CoA reductase activity in control chicks were practically similar to those observed in the present work. When control animals were euthanized 6 hours after food consumption a near twofold activity was found.[29,38] This high level was also maintained during further development (21–28 days).[41] A similar inhibition of reductase activity by cholesterol feeding also was observed in chick intestine[40] and in isolated enterocytes from chick duodenum, jejunum, and ileum,[42] although in this tissue differences between peak and nadir values were always smaller than those observed in liver.[43]

The hypercholesterolemic effect of cholesterol was generally accompanied by the accumulation of cholesterol in liver, thus interfering with the hepatic cholesterogenesis. In fact, dietary cholesterol supplementation to the newborn chick after 10 to 15 days produced a near 10-fold increase in the hepatic esterified cholesterol, whereas no significant variations were found in free cholesterol. The same dietary manipulation induced a significant inhibition not only of HMG-CoA reductase activity[40] but also of mevalonate incorporation into total nonsaponifiable lipids,[17,35] as well as in mevalonate 5-pyrophosphate decarboxylase activity.[44] A fraction of lanosterol derivatives and cholesterol precursors accumulated after cholesterol feeding may be responsible for the HMG-CoA reductase inhibition[45,46] as well as for the decrease of cholesterol synthesis from mevalonate.[46] Diminution of these nonsaponifiable lipids in chick liver between 5 and 11 days of postnatal development also may explain the clear increase of HMG-CoA reductase activity found during the same neonatal period.[47] It has been reported that cholesterol biosynthesis is suppressed in mammalian cells by the addition of whole serum to the culture medium.[48] This suppression, which is mediated by cholesterol, is reversible and appears to be caused by a decrease in the synthesis of HMG-CoA reductase.[49] Our recent results show a similar decrease in decarboxylase activity by serum lipoproteins.[50] Therefore, the strong inhibition of hepatic HMG-CoA reductase activity may be due to the clear increase found in hepatic cholesterol levels after coconut oil plus cholesterol feeding. The lack of HMG-CoA reductase activity response to coconut oil feeding also may be related to the absence of significant changes induced in hepatic cholesterol content by the same dietary treatment.

The mechanisms involved in the hypocholesterolemic effects of polyunsaturated fat have not been clearly defined. An increase in hepatic LDL receptors has been proposed.[36] However, the possibility that lower levels of circulating cholesterol are achieved through an inhibitory effect of n-3 PUFA on HMG-CoA reductase also has been suggested. In different animal models, some investigators have observed a decrease in cholesterol synthesis from consumption of PUFA compared with saturated fat,[51] whereas others have found no significant difference in endogenous cholesterol production rates.[52,53] More recently, Mizuguchi et al.[54] 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, probably due to the increase in microsomal free cholesterol.[54] However, results obtained in our laboratory seem to indicate that no significant differences were observed in microsomal cholesterol after supplementation of menhaden oil to the diet,[39] in contrast to the rapid increase of cholesterol and the cholesterol/phospholipid molar ratio found in chick microsomes after cholesterol feeding.[38] Changes in fatty acid composition of microsomal phospholipids may be related to the inhibition of HMG-CoA reductase found after menhaden oil administration (unpublished data).

Putative mechanisms underlying the major metabolic effects of fish oil include an increase in plasma lipid clearance and a decrease in lipid biosynthesis. Thus, it has been reported that EPA-E probably causes a rapid conversion of VLDL to intermediate density lipoprotein and LDL and/or induces its rapid absorption into the liver, accounting for the rapid clearance of VLDL shown in rats.[55] Our results (see *Table 4*) of the transient depletion of chick hepatic cholesterol after 1 week of n-3 PUFA supplementation to the diet are in agreement with those obtained with other inhibitors of cholesterol synthesis. Recent data from Ness et al.[56] indicate that administration of HMG-CoA reductase inhibitors resulted in increased hepatic LDL receptor mRNA levels, suggesting an enhanced hepatic uptake of lipoproteins without increasing steady-state levels of LDL

receptor protein. More studies would be necessary to obtain conclusive information on the effects of fish oil on chick lipoprotein metabolism, especially on the VLDL fraction, bearing in mind the important role of this fraction in avian atherogenesis and its rapid response to coconut oil administration.[20]

In summary, data presented in this article suggest the existence of important differences in the regulatory mechanisms implied in cholesterol biosynthesis and its accumulation in plasma by the different nutrients. Thus, PUFA and cholesterol that showed the same inhibitory effect on HMG-CoA reductase activity produced opposite effects on plasma cholesterol content, whereas saturated fatty acids and cholesterol that induced a clear accumulation of plasma cholesterol showed a different effect on the rate of cholesterogenesis, which are measured as variations in the HMG-CoA reductase activity.

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