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Coconut Oil Affects Lipoprotein Composition and Structure of Neonatal Chicks[1]

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Supplementation of 10 or 20% coconut oil in the diet for 1-2 weeks produced a significant hypercholesterolemia in neonatal chicks. Plasma triacylglycerol concentration significantly increased after the addition of 20% coconut oil for 2 weeks. These results show that newborn chicks are more sensitive to saturated fatty acids from coconut oil than adult animals. The effects of this saturated fat on lipoprotein composition were studied for the first 1-2 weeks of neonatal chick life. Coconut oil supplementation in the diet (20%) for 2 weeks increased cholesterol concentration in all the lipoprotein fractions, while 10% coconut oil only increased cholesterol in low-density and very-low-density lipoproteins, an increase that was significant after 1 week of treatment. Similar results were obtained for triacylglycerol concentration after 2 weeks of treatment. Changes in phospholipid and total protein levels were less profound. Coconut oil decreased low-density and very-low-density lipoprotein fluidity, measured as total cholesterol/phospholipid ratio. Changes in esterified cholesterol/phospholipid and triacylglycerol/phospholipid ratios suggest that coconut oil affects the distribution of lipid components in the core of very-low-density particles. Likewise, the esterified cholesterol/triacylglycerol ratio was clearly increased in the low-density, and especially in the very-low-density, fraction after the first week of coconut oil feeding. Our results show that neonatal chick provides a suitable model in which to study the role of very-low-density lipoproteins in atherogenesis and the rapid response to saturated fatty acids with 12-14 carbons.

Key words: atherogenesis, coconut oil, hypercholesterolemia, lipoprotein, neonatal chick.

The role of blood plasma cholesterol as a factor in stimulating atherogenesis is now widely accepted. Plasma cholesterol concentration is strongly influenced by the quantity and quality of fat in the diet. Cholesterol and saturated fat increase total plasma cholesterol concentration. Although most research has focused on the effects of diet on serum total cholesterol concentration, in recent years attention has shifted to individual lipoproteins, *i.e.*, low-density lipoproteins (LDL), high-density lipoproteins (HDL), and very-low-density lipoproteins (VLDL) (*1*).

The ability of dietary saturated fatty acids to raise total cholesterol levels, compared to carbohydrate, is well established. Early reports (*2-4*) indicating that saturated acids increase total cholesterol concentration have not been contradicted by subsequent investigations. Moreover, later reports (*5-7*) indicate that LDL-cholesterol level, as well as total cholesterol concentration, is raised by saturated fat in the diet. However, recent findings by other investigators (*8, 9*) indicate that saturated fatty acids can not be regarded as a single entity, because differences in the chain length seem to influence plasma cholesterol concentration differently.

The chick has been recognized as a suitable animal model for studies on the comparative biochemistry of cholesterol metabolism and transport because this animal is highly sensitive to dietary cholesterol (*10*). Thus, we have found that diet supplementation with 2% cholesterol produced a significant hypercholesterolemia in 30-day-old chicks after 3 days of treatment (*11*). The hypercholesterolemic effect of cholesterol was generally accompanied with an accumulation of cholesterol in liver, interfering with hepatic cholesterogenesis by causing a drastic decrease in the hepatic 3-hydroxy-3-methylglutaryl-CoA reductase activity (*12*). Likewise, the induction of experimental hypercholesterolemia in 14-day-old chicks by saturated fat has recently been reported (*13*).

The chick emerges from the egg with a large deposit of cholesterol in three tissues: yolk sac, liver, and plasma (*14, 15*). Cholesterol accumulated in liver and plasma was mainly in the esterified form (*14*), suggesting a massive transfer of lipids from the yolk sac to the emerging chick. It is also well established that within a few days after hatching there is a progressive depletion of cholesterol in chick liver (*16*) and plasma (*17*). These rapid changes of cholesterol content in different tissues render the newborn chick an interesting model for the study of cholesterol metabolism throughout postnatal development.

Although there have been no systematic studies on

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Abbreviations: HDL, high-density lipoproteins; IDL, intermediate-density lipoproteins; LDL, low-density lipoproteins; VLDL, very-low-density lipoproteins.

plasma lipoprotein of the developing chick, we have reported that serum total cholesterol clearly decreased between 4 and 7 days after hatching, with important changes in the chemical composition of different lipoprotein classes (*17*). Thus, the amount of total lipoprotein markedly decreased between 4 and 7 days after hatching, reaching a practically constant level from 14 days onwards. Both VLDL and intermediate density lipoproteins (IDL) represented about 10% of total lipoproteins in the newborn chick. Both percentages clearly decreased from 7 days onwards. Cholesterol content of both VLDL and IDL fractions strongly decreased from 1 to 7 days. Changes in LDL-cholesterol were less profound, while HDL-cholesterol remained unaltered during the first 14 days.

The effect of 2% dietary cholesterol on the distribution of cholesterol among the plasma lipoproteins has been studied recently in 14-day-old chicks (*18*). In our laboratory, we have also studied the effect of dietary coconut oil on lipoprotein composition of young chicks of a similar age (*19*). Bearing in mind the extensive changes in the lipid composition and metabolism of chick plasma, liver and other tissues, we have studied the effect of 10 or 20% coconut oil supplementation in the diet through the neonatal period on the chemical composition of different chick lipoprotein fractions.

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 09:00 to 21:00 h and a controlled temperature (28°C). Control animals were fed on a commercial diet free of cholesterol (Sanders A-00, Granada, Spain) which contained (w/w) 45.2% carbohydrate (mainly starch), 3.5% fat, and 20.5% protein. Experimental diets were prepared by supplementation with 10 or 20% (w/w) coconut oil in the standard diet from hatching. All animals had free access to water and food. Fatty acid compositions of the analyzed diets are given in Table I.

After 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 2,500 rpm for 20 min at 4°C. Lipoprotein fractions were isolated by density gradient ultracentrifugation as previously described (*20*). The stability of the gradient was maintained by collecting the different fractions from the bottom with a hypodermic needle attached to a peristaltic pump, instead of from the meniscus of each tube by aspiration with a narrow-bore Pasteur pipette.

The following components were determined in each fraction: total cholesterol and triacylglycerols by enzymatic colorimetric methods by using "Test-Combination cholesterol" or "GPO-PAP Test," respectively, from Boehringer Mannheim GmbH, (Mannheim, Germany); phospholipids by the method of Bartlett (*21*) and protein by the method of Lowry *et al.* (*22*) using bovine albumin as a standard.

Three experiments were performed in each case. Equal amounts of plasma from 6 animals were pooled for each of the three experiments. Triplicate determinations were carried out in each experiment. Student's *t* test was used to compare the values obtained from control and treated animals.

RESULTS

Coconut oil supplementation in the diet (10 or 20%) did not significantly interfere with the growth rate of animals. Feed intake was similar in all the animals.

Supplementation of 10 or 20% coconut oil in the diet produced a clear hypercholesterolemia in neonatal chicks. As can be seen in Table II, the level of total cholesterol was significantly increased after 1 week of 10% coconut oil treatment. Addition of 20% coconut oil produced a greater increase in total cholesterol level than that observed after 10% coconut oil supplementation. Triacylglycerol concentration significantly increased only after addition of 20% coconut oil for 2 weeks.

Chick lipoprotein fractions were obtained as described in Ref. *20* and analyzed by electrophoresis on discontinuous polyacrylamide gel film. This analysis revealed the presence of three main bands (Fig. 1). From a comparison with the electrophoretic mobilities of lipoproteins from chick and other sources (*23*) and according to their chemical composition and density profile, fractions 20-19 were considered to behave as VLDL, fractions 17-16 as IDL, fractions 14-12 as LDL, and fractions 6-5 as HDL. In other

TABLE I. **Fatty acid composition of the experimental diets.** Results are expressed as mean values (%) of three determinations. SEM < 15%.

Fatty acid	Diets		
	Standard	+10% coconut oil	+20% coconut oil
10	–	0.3	0.6
12	–	5.2	10.5
14	0.7	2.7	4.8
16	22.3	21.1	20.1
16-1(*n*-7)	3.2	2.9	2.5
18	8.5	8.7	9.1
18:1(*n*-9)	32.2	29.0	25.7
18:2(*n*-6)	2.3	2.1	1.8
18:3(*n*-3)	24.6	22.1	19.7
20:2(*n*-6)	2.3	2.1	1.8
20:3(*n*-6)	0.8	0.7	0.6
20:4(*n*-6)	1.3	1.2	1.0
20:5(*n*-3)	1.5	1.3	1.2
Others	0.3	0.6	0.6
Σ saturated	31.5	38.0	45.1
Σ unsaturated	68.2	61.4	54.3
Satur./unsatur.	0.46	0.62	0.83

TABLE II. **Effects of coconut oil on total cholesterol and triacylglycerol levels in chick plasma.** Results are expressed as mean values ± SEM of three experiments carried out with pools of 6 animals. Triplicate determinations were made in each experiment. Statistical significance is indicated by $^{a}p<0.05$; $^{b}p<0.005$, or $^{c}p<0.0005$ with respect to the control.

	Component (mg/ml plasma)	
	Total cholesterol	Triacylglycerol
1st week		
Control	1.62±0.04	0.43±0.04
+10% coconut oil	1.98±0.03[a]	0.50±0.03
+20% coconut oil	2.47±0.10[b]	0.51±0.05
2nd week		
Control	1.70±0.06	0.49±0.03
+10% coconut oil	2.16±0.04[b]	0.60±0.04
+20% coconut oil	3.29±0.12[b]	1.46±0.02[c]

fractions, the amounts of material were too small to be detected by lipid staining.

Electrophoresis of the protein moieties of fractions of chick VLDL, IDL, and LDL in 4% SDS polyacrylamide gel slabs showed each to possess a protein with a molecular weight of 400,000-450,000 as its major component. This apoprotein appears to correspond to the major form of chick apolipoprotein B described by other authors (*23, 24*). The electrophoretic patterns of apolipoproteins of chick VLDL, IDL, LDL, and HDL were examined in a discontinuous gradient of 8-18% SDS-polyacrylamide gel slabs. Figure 2 shows that a band with a molecular weight of about 27,000 was detected in all fractions. This band was present in large amounts in fraction 14, corresponding to LDL, as described by Hermier *et al.* (*23*), and presented the same mobility as human apolipoprotein A. Chick fractions corresponding to VLDL, LDL, and HDL also showed an apolipoprotein band with a molecular weight of about 21,000, similar to that previously found in chick (*23*). As was expected, two bands with molecular weights about 13,000 and 11,000 were detected in HDL, LDL, and VLDL. An additional band with a molecular weight of about 45,000, probably representing a chicken counterpart of human apolipoprotein A-IV (*23*), was faintly stained in fraction 14, corresponding to LDL.

The effects of 10 or 20% coconut oil on chick lipoprotein composition were studied after 1 or 2 weeks of treatment. Table III shows that 20% coconut oil supplementation in the diet for 2 weeks produced a significant increase of cholesterol level in all the lipoprotein fractions, while 10% coconut oil only increased LDL- and VLDL-cholesterol, an

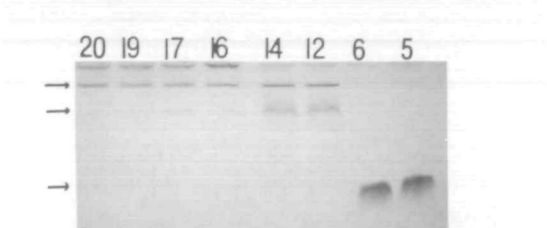

Fig. 1. **Electrophoresis of lipoprotein gradient fractions on discontinuous polyacrylamide gel film ("Lipofilm").** Samples were prestained with Sudan black and electrophoresed in parallel. Fractions (20, 19 VLDL; 17, 16 IDL; 14, 12 LDL; 6, 5 HDL) were applied in order from left to right. The arrows indicate the respective lipoprotein bands

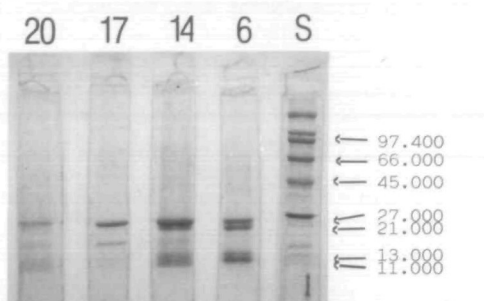

Fig 2 **Electrophoretic patterns in 8-18% SDS polyacrylamide gel slabs of chick apolipoproteins from gradient fractions.** For comparative purposes, standard molecular weight markers (lane S) were electrophoresed in parallel The arrows indicate the major protein bands in chick lipoprotein fractions, with the corresponding molecular weights

TABLE III. **Effects of coconut oil on cholesterol concentration in different lipoprotein fractions from chick plasma.** Results are expressed as in Table II Statistical significance is indicated by $^{a}p<0.05$, $^{b}p<0.005$, or $^{c}p<0.0005$ with respect to the control

	HDL	LDL	IDL	VLDL
	(mg/ml plasma)			
1st week				
Total cholesterol				
Control	0.934±0 083	0.365±0.026	0.149±0 029	0.088±0.001
+10% coconut oil	1.111±0 073	0.529±0.010[a]	0.144±0.022	0.145±0 008[b]
+20% coconut oil	1.131±0 034	0.664±0.011[c]	0.124±0 023	0.145±0.010[b]
Free cholesterol				
Control	0.239±0 013	0.162±0.026	0.055±0.004	0.051±0.007
+10% coconut oil	0.311±0 009[a]	0.200±0.003	0.053±0.007	0.044±0 005
+20% coconut oil	0.308±0 003	0.239±0.001[a]	0.052±0.006	0.064±0.014
Esterified cholesterol				
Control	0.695±0.057	0.203±0.015	0.094±0.031	0.037±0 005
+10% coconut oil	0.800±0 088	0.329±0 029[a]	0.091±0.025	0.101±0.010[b]
+20% coconut oil	0.823±0.037	0.425±0.012[c]	0.072±0.029	0.081±0.014[a]
2nd week				
Total cholesterol				
Control	1.036±0 038	0.417±0.006	0.081±0.004	0 060±0 006
+10% coconut oil	1.099±0 061	0.625±0.004[a]	0.101±0.016	0.110±0.006[b]
+20% coconut oil	1 453±0 010[c]	1.298±0 038[c]	0.195±0.011[b]	0 130±0.011[a]
Free cholesterol				
Control	0.275±0 005	0.167±0.015	0.032±0.003	0 023±0.001
+10% coconut oil	0.290±0.019	0.210±0.021	0.045±0.002[a]	0.048±0.007[a]
+20% coconut oil	0 374±0.026[a]	0.533±0.003[c]	0.103±0.010[b]	0 045±0.003[c]
Esterified cholesterol				
Control	0 761±0 025	0.250±0 041	0.049±0.006	0.037±0.008
+10% coconut oil	0.809±0 081	0.415±0.035[a]	0.056±0.017	0 062±0.004[a]
+20% coconut oil	1.059±0 026[a]	0.765±0.041[b]	0.092±0.017	0.085±0.014[a]

increase that was significant after 1 week of treatment. In both LDL and VLDL, changes in the esterified cholesterol were more pronounced than those observed in the free form. When 20% coconut oil supplementation in the diet was prolonged for 2 weeks, both free and esterified cholesterol concentrations were greater than those found in control animals in all the lipoprotein fractions. Similar increases were obtained in triacylglycerol (Table IV), but in this case the effects were significant only after 2 weeks of treatment. Changes in phospholipid levels were less profound. As can be seen in Table V, only LDL and IDL fractions were significantly affected in their phospholipid content by 20% coconut oil supplementation in the diet for 2 weeks. Similar changes were observed in total protein levels (Table VI).

The atherogenic index, measured as LDL-cholesterol/HDL-cholesterol ratio, increased in relation to the percentage of saturated fat supplemented in the diet and the assay duration: 0.587±0.020 *versus* 0.391±0.045 after 1 week of 20% coconut oil supplementation, and 0.569±0.022 (10% coconut oil) and 0.893±0.026 (20% coconut oil) *versus* 0.402±0.015 after 2 weeks of treatment.

In order to establish a relationship between changes in lipoprotein composition and structure as a consequence of this saturated fat feeding, we have compared in detail different lipid ratios in each fraction. All the lipid ratios

TABLE IV. **Effects of coconut oil on triacylglycerol concentration in different lipoprotein fractions from chick plasma.** Results are expressed as in Table II. Statistical significance is indicated by $^{a}p<0.05$; $^{b}p<0.005$, or $^{c}p<0.0005$ with respect to the control.

	HDL	LDL	IDL	VLDL
		(mg/ml plasma)		
1st week				
Control	0.204±0.015	0.095±0.004	0.036±0.002	0.080±0.013
+10% coconut oil	0.208±0.003	0.105±0.010	0.031±0.006	0.093±0.005
+20% coconut oil	0.226±0.014	0.120±0.007^{a}	0.025±0.007	0.085±0.018
2nd week				
Control	0.182±0.013	0.130±0.007	0.028±0.004	0.075±0.010
+10% coconut oil	0.179±0.004	0.185±0.040^{a}	0.033±0.003	0.112±0.013
+20% coconut oil	0.378±0.051^{a}	0.265±0.003^{c}	0.068±0.005^{b}	0.239±0.026^{b}

TABLE V. **Effects of coconut oil on phospholipid concentration in different lipoprotein fractions from chick plasma.** Results are expressed as in Table II. Statistical significance is indicated by $^{a}p<0.05$ or $^{b}p<0.005$ with respect to the control.

	HDL	LDL	IDL	VLDL
		(mg/ml plasma)		
1st week				
Control	1.690±0.080	0.525±0.085	0.180±0.050	0.082±0.005
+10% coconut oil	1.940±0.080	0.620±0.020	0.130±0.040	0.070±0.010
+20% coconut oil	1.860±0.060	0.710±0.020	0.100±0.060	0.075±0.010
2nd week				
Control	2.330±0.040	0.660±0.030	0.095±0.005	0.058±0.015
+10% coconut oil	2.615±0.005	0.780±0.010	0.110±0.010	0.075±0.010
+20% coconut oil	2.870±0.010	1.020±0.040^{b}	0.200±0.020^{a}	0.085±0.040

TABLE VI. **Effects of coconut oil on protein concentration in different lipoprotein fractions from chick plasma.** Results are expressed as in Table II. Statistical significance is indicated by $^{a}p<0.05$ or $^{b}p<0.005$ with respect to the control.

	HDL	LDL	IDL	VLDL
		(mg/ml plasma)		
1st week				
Control	2.330±0.660	0.615±0.085	0.145±0.025	0.072±0.012
+10% coconut oil	2.650±0.390	0.780±0.090	0.130±0.020	0.080±0.015
+20% coconut oil	2.270±0.380	0.790±0.140	0.120±0.050	0.070±0.010
2nd week				
Control	2.445±0.075	0.645±0.065	0.110±0.010	0.055±0.020
+10% coconut oil	2.630±0.190	0.815±0.005	0.120±0.010	0.043±0.003
+20% coconut oil	2.900±0.130	1.230±0.020^{b}	0.200±0.001^{a}	0.075±0.005

TABLE VII. **Effects of coconut oil on different lipid ratios in LDL fraction from chick plasma.** Results were taken from Tables III-V for LDL fraction. Statistical significance is indicated by $^{a}p<0.05$; $^{b}p<0.005$; $^{c}p<0.0005$.

	TC/PL	EC/PL	EC/TG	FC/PL	TG/PL
1st week					
Control	0.695±0.024	0.387±0.038	2.137±0.181	0.308±0.070	0.181±0.030
+10% coconut oil	0.853±0.037^{a}	0.531±0.033^{a}	3.134±0.144^{a}	0.323±0.011	0.169±0.017
+20% coconut oil	0.935±0.030^{b}	0.599±0.016^{a}	3.542±0.106^{b}	0.336±0.058	0.169±0.050
2nd week					
Control	0.794±0.030	0.379±0.034	1.923±0.331	0.253±0.025	0.197±0.014
+10% coconut oil	0.801±0.012	0.532±0.028^{a}	2.243±0.153	0.269±0.027	0.237±0.043
+20% coconut oil	1.273±0.062^{c}	0.750±0.002^{b}	2.887±0.004^{a}	0.522±0.001^{c}	0.260±0.052

TABLE VIII. **Effects of coconut oil on different lipid ratios in VLDL fraction from chick plasma.** Results were taken from Tables III-V for VLDL fraction. Statistical significance is indicated by $^{a}p<0.05$.

	TC/PL	EC/PL	EC/TG	FC/PL	TG/PL
1st week					
Control	1.073±0.066	0.451±0.066	0.462±0.097	0.621±0.093	0.976±0.164
+10% coconut oil	2.071±0.298[a]	1.442±0.223[a]	1.086±0.131[a]	0.628±0.114	1.328±0.202
+20% coconut oil	1.933±0.265[a]	1.080±0.178[a]	0.953±0.033[a]	0.853±0.218	1.133±0.283
2nd week					
Control	1.034±0.286	0.638±0.215	0.493±0.125	0.396±0.103	1.293±0.376
+10% coconut oil	1.467±0.202	0.827±0.162	0.554±0.142	0.640±0.126	1.493±0.263
+20% coconut oil	1.529±0.226	1.000±0.273	0.356±0.171	0.529±0.149	2.812±0.634

studied were maintained practically constant after the treatments in the HDL and IDL fractions. However, the chemical composition of LDL and VLDL showed different behavior in response to coconut oil. Thus, although the fluidity of lipoproteins depends on many biochemical factors, the total cholesterol/phospholipid ratio (TC/PL), which has been considered as an inverse index of membrane fluidity, was clearly increased in LDL (Table VII) and VLDL particles (Table VIII) by the dietary regimens after the first week of treatment, while after more prolonged feeding (2 weeks) this ratio seemed to revert to the control values, especially in the VLDL fraction. These results demonstrate that dietary coconut oil rapidly decreased the fluidity of LDL and VLDL fractions. Likewise, esterified cholesterol/phospholipid ratio (EC/PL) was significantly increased in both fractions by the dietary manipulations during the first week. Similar changes were found in the esterified cholesterol/triacylglycerol ratio (EC/TG) in LDL and VLDL fractions, especially after the first week of dietary manipulation.

DISCUSSION

The increase in plasma cholesterol observed after coconut oil supplementation in the diet in newborn chicks corroborates previous observations by many investigators in other animal species (*2-4*). However, it is important to note that the effect of 10% coconut oil on neonatal animals was significant after the first week of treatment, while in 14-day-old chicks this effect was not significant under the same conditions (*13*). Likewise, 2% cholesterol supplementation in the diet for 1 week was not sufficient to produce a significant hypercholesterolemia in neonatal chicks (*11*). These results suggest that newborn chicks are more sensitive to saturated fatty acids with 12-14 carbons than adult animals and less sensitive to dietary cholesterol, probably as a consequence of the transient hypercholesterolemia presented in the newborn chicks.

Although the chick, like cebus monkey, is primarily an HDL animal with respect to cholesterol transport, the elevation in plasma lipid caused by saturated fat feeding is largely the result of increased LDL and VLDL pools, irrespective of the major circulating lipoprotein class (*25*). The mechanisms whereby certain saturated fatty acids raise LDL levels are not completely understood. Studies in humans indicate that this action is due mainly to impaired removal of LDL from the circulation (*1*). Loo *et al.* (*26*) have suggested that dietary cholesterol does not suppress the number of LDL binding sites in cockerel liver, in sharp contrast to the effect of dietary cholesterol on mammalian liver LDL receptors. However, it is not known if true LDL or HDL receptors exist in the liver of cockerels (*26*). It has been proposed that the laying hen expresses two different LDL receptor proteins (*27*). One of these, termed VLDL/VTG receptor (VLDL/VTGR) is a 95 kDa plasma membrane protein exclusively produced in oocytes, and binds two precursors of yolk, VLDL and vitellogenin. *In vitro*, this receptor also binds apoE, which is not synthesized by avian species. Vitellogenin could be a counterpart of mammalian apo E. The other receptor, 130 kDa, seems to be restricted to somatic cells and presents all the biochemical characteristics of the mammalian LDL receptor. The laying hen also contains a pair of large proteins akin to mammalian LDL receptor-related protein (LRP) (*28*). The tissue distribution of these two proteins is compatible with one being oocyte-specific and the other somatic cell-specific (*29*). Recently (*30*), molecular characterization of the VLDL/VTGR from chicken oocytes revealed that it is a member of the LDL receptor gene superfamily that is expressed almost exclusively in oocytes.

Likewise, it has been reported that coconut oil feeding alone was not sufficient to induce a significant reduction in hepatic LDL receptor mRNA levels in cebus monkey (*25*). The fact that the VLDL plus LDL cholesterol levels of these animals fed coconut oil without cholesterol, relative to those fed corn oil alone, were dramatically increased suggests that the influence of dietary fatty acid composition may be considerable with respect to LDL receptor activity, independently of production. Coconut oil consumption may enhance the incorporation of saturated fatty acids into the LDL receptor membrane and, ultimately, inhibit its function (*31*).

On the other hand, the reported effects of different nutrients on HDL are contradictory. Kruski and Narayan (*32, 33*) reported that HDL cholesterol was lower after 2-4 weeks of cholesterol feeding to chickens, while Loo *et al.* (*26*) found a significant increase in HDL cholesterol in cholesterol-supplemented cockerels at 11 weeks. Recent studies in 14-day-old chicks (*18*) have established that there is no direct explanation for the significant decrease in HDL which was found in cholesterol-fed chickens. It is noteworthy that birds are not protected against atherosclerosis, despite considerable amounts of plasma HDL. The rational behind this may be that HDL without apoE cannot be involved in the reverse cholesterol transport (*18*).

Our results show a marked increase in VLDL cholesterol in chicks fed coconut oil for 1-2 weeks. These results are in agreement with the findings of several authors (*22, 32, 33*) in cockerels fed a cholesterol-supplemented diet, so that the effect of coconut oil on these lipoprotein fractions was similar to that found after cholesterol feeding. Likewise,

the hypercholesterolemia of the newborn chick was mainly due to the accumulation of cholesterol-rich VLDL and IDL (*17*). Recent studies by Hermier and Dillon (*18*) also showed that the VLDL fraction was enriched in cholesterol when young birds were fed a cholesterol-rich diet for 5 weeks. As in our experiments, chicks were fasted overnight and it is, therefore, likely that VLDL represented the form in which dietary cholesterol is reassociated with lipoprotein particles and secreted by the liver (*18*). The relative increase in the proportion of cholesterol in VLDL and IDL fractions found when young birds were fed the cholesterol-rich diet was systematically accompanied with a decrease in triacylglycerol content. Consequently, the average sum of triacylglycerol plus cholesteryl esters, which essentially represents the hydrophobic core of the particle, remained remarkably constant in both VLDL and IDL-LDL in control and hypercholesterolemic birds (*18*). On the contrary, in our experiments triacylglycerol contents in VLDL and IDL fractions were significantly increased after 2 weeks of 20% coconut oil supplementation in the diet.

The study of different lipid ratios in each lipoprotein fraction after coconut oil feeding may be important because it has been suggested that the physicochemical structure of these particles may play an important role in their atherogenicity. Thus, human lipid nutrition studies have shown that LDL from subjects fed polyunsaturated fat as a hypocholesterolemic diet are more fluid than those from subjects fed a saturated fat diet (*34, 35*). Our results show that coconut oil decreased the fluidity not only in LDL but also in VLDL fraction. Fluorescence polarization studies corroborated this effect (results not shown). Changes in VLDL fraction were more patent in the EC/PL ratio than in the FC/PL ratio, suggesting that saturated fat drastically affects the distribution of lipid components between the core of particle (esterified cholesterol and triacylglycerol molecules) and the surface. This view is supported by a comparison of the TG/PL ratio in the VLDL fraction. Finally, the EC/TG ratio was clearly increased in LDL and, especially, in VLDL fractions after the first week of coconut oil feeding, corroborating the main atherogenic function of this lipoprotein fraction and the rapid response to coconut oil in neonatal chick, suggesting that the physicochemical properties of the core of these lipoproteins can be modified.

More studies would be necessary to obtain conclusive information on the role of different lipoprotein classes in the alterations of the chick plasma lipoprotein profile in different nutritional conditions, but the accumulation of VLDL in diet-induced hypercholesterolemia suggests an important role of this fraction in avian atherogenesis. Therefore, coconut oil feeding to neonatal chick provides a suitable model in which to study the role of VLDL in atherogenesis and the rapid response to this saturated fat.

Finally, it is interesting to see that this animal has at its disposal at least four receptor proteins known to be the products of four different but related genes, providing an exciting scenario to unravel the molecular basis for differentiated functions and cell-specific expression of LDLR gene family members. One of the two cell-specific receptor systems ensures systemic lipid (cholesterol) homeostasis through its expression in the somatic cells, and the other system mediates lipoprotein (and triglyceride) transport to the oocyte. The subtle mechanisms which can simultaneously maintain cholesterol homeostasis in somatic cells and massive transport of lipids to the growing chicken oocyte, as well as their biological significance in lipoprotein metabolism, are far from elucidation (*36*).

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