# Effects of diet on chick plasma and lipoprotein composition. Influence of age

## Efecto de la dieta sobre la composición química del plasma y de las lipoproteínas plasmáticas de pollo recién nacido. Influencia de la edad

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**ABSTRACT**

The effects of cholesterol and/or coconut oil supplementation to the diet on plasma and lipoprotein composition have been studied in neonatal and young chicks. Supplementation of 2% cholesterol produced a significant hypercholesterolemia in young chicks after 3 days of treatment, while in newborn chicks the same effect was observed only after 15 days of treatment. However, coconut oil supplementation to the diet (10 or 20%) produced a significant hypercholesterolemia after 7 days of dietary manipulation both in neonatal and young chicks. Contrary to that observed after cholesterol feeding, the hypercholesterolemic effect of coconut oil was not accompanied by changes in the levels of liver cholesterol. Coconut oil mainly incresed the cholesterol content in HDL and LDL lipoprotein fractions in young chicks, while in newborn animals the increases were mainly found in LDL- and VLDL-cholesterol. Simultaneous supplementation of coconut oil (10%) plus cholesterol (1%) to the diet drastically increased the levels of plasma and liver cholesterol, as well as in VLDL.

**Key words**: Cholesterol. Coconut oil. Lipoprotein. Neonatal and young chick.

**RESUMEN**

Se ha estudiado el efecto de la suplementación a la dieta con colesterol y/o aceite de coco (rico en ácidos grasos saturados 14:0 y 12:0) sobre la composición del plasma y de las lipoproteínas de pollo neonato y joven. El colesterol añadido al 2% produce una significativa hipercolesterolemia a los 3 días de tratamiento en los pollos jóvenes, mientras que en los recién nacidos se necesitan 15 días para observar el mismo efecto. La adición de aceite de coco (al 10 y 20%) produce una hipercolesterolemia significativa a los 7 días de manipulación dietaria, tanto en los animales neonatos como en los jóvenes. Al contrario de lo observado tras la adición de colesterol, el aceite de coco no afecta a los niveles de colesterol en el hígado. A nivel de las lipoproteinas, esta grasa saturada incrementa sobre todo los niveles de colesterol en las fracciones HDL y LDL en los pollos jóvenes y LDL y VLDL en los recién nacidos. La adición conjunta de aceite de coco (10%) y colesterol (1%) aumenta drásticamente los niveles de colesterol en plasma y en hígado, así como en la fracción VLDL.

**Palabras clave**: Colesterol. Aceite de coco. Lipoproteinas. Pollo recién nacido y joven.

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## INTRODUCTION

Diets rich in saturated fat and/or cholesterol are associated with the development of coronary heart disease in man and animals. However, several studies have shown that the increase of plasma lipids in response to dietary factors is highly variable even among normolipidemic subjects (1,2). The chick has been recognized for some years 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 (3). The chick emerges from the egg with a large deposit of cholesterol in yolk sac, liver and plasma. Cholesterol accumulated in liver and plasma was mainly in the esterified form (4), 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 (5) and plasma (6). 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.

Bearing in mind these considerations, in the present work we have studied the effects of cholesterol and/or saturated fat (coconut oil, rich in 14:0 and 12:0 fatty acids) on plasma and lipoprotein cholesterol content in neonatal and young chicks.

## 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). Control animals were fed ad libitum on a commercial diet (Sanders A-00). Supplementation with 2% cholesterol and 10-20% coconut oil started after hatching in the newborn animals and 1 h before the light period on day 14 of life in the young animals. The coconut oil was for pharmaceutical use (Acofarma). 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. Supplementation of 2% cholesterol to the standard diet did not change significantly the fatty acid composition of this diet.

After each treatment, blood was taken from each animal be decapitation

Table 1.—Fatty acid composition of diets

| *Fatty acid* | *Control* | *CO10* | *CO20* |
|---|---|---|---|
| 8:0 | | 1.7 | 2.2 |
| 10:0 | | 3.0 | 3.7 |
| 12:0 | | 29.9 | 37.3 |
| 14:0 | 0.8 | 12.4 | 15.1 |
| 16:0 | 22.3 | 14.9 | 13.0 |
| 18:0 | 8.6 | 5.6 | 4.8 |
| Total Sat. | 31.7 | 67.5 | 76.1 |
| 16:1 n-7 | 3.3 | 1.3 | 0.8 |
| 18:1 n-9 | 32.4 | 17.4 | 13.7 |
| Total MUFA | 35.7 | 18.7 | 14.5 |
| 18:3 n-3 | 0.8 | 0.3 | 0.2 |
| 22:5 n-3 | 1.7 | 0.7 | 0.4 |
| Total n-3 | 2.5 | 1.0 | 0.6 |
| 18:2 n-6 | 24.6 | 10.8 | 7.4 |
| 20:2 n-6 | 2.5 | 1.0 | 0.6 |
| 20:3 n-6 | 1.1 | 0.5 | 0.3 |
| 20:4 n-6 | 1.6 | 0.6 | 0.4 |
| Total n-6 | 29.8 | 12.9 | 8.7 |
| Sat./Unsat. | 0.46 | 2.07 | 3.20 |
| Sat./PUFA | 0.98 | 4.85 | 8.18 |
| n-3/n-6 | 0.08 | 0.08 | 0.07 |

CO10, diet supplemented with 10% coconut oil; CO20, diet supplemented with 20% coconut oil.

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. Lipoprotein fractions were isolated by density gradient ultracentrifugation as previously described (7). The following components were determined in each fraction as follows: total and free cholesterol as well as triacylglycerol contents by enzymic colorimetric methods using the respective commercial tests from Boehringer Mannheim, Mannheim, Germany; phospholipids by the method of Bartlett (8) and protein by the method of Lowry et al. (9).

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.

Table 3.—Effect of coconut oil feeding on cholesterol levels in neonatal and young chick plasma Component (mg/ml plasma)

| | | *Total cholesterol* | | | *Free cholesterol* | | | *Esterified cholesterol* | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Days of treatment* | *C* | *CO10* | *CO20* | *C* | *CO10* | *CO20* | *C* | *CO10* | *CO20* |
| Neonatal chick | 7 | 1.50±0.03 | 1.91±0.04[a] | 2.12±0.02[b] | 0.57±0.02 | 0.75±0.01[a] | 0.77±0.01[a] | 0.93±0.03 | 1.16±0.05[b] | 1.35±0.04[b] |
| | 14 | 1.69±0.06 | 2.12±0.03[b] | 3.36±0.11[b] | 0.46±0.03 | 0.60±0.02[a] | 1.25±0.02[c] | 1.23±0.06 | 1.52±0.07[a] | 2.11±0.08[c] |
| Young chick | 7 | 1.77±0.04 | 2.11±0.06[a] | 2.91±0.15[b] | 0.54±0.01 | 0.63±0.01[b] | 1.16±0.13[b] | 1.23±0.04 | 1.48±0.24 | 1.75±0.19[a] |
| | 14 | 1.71±0.05 | 2.52±0.07[c] | 3.27±0.03[c] | 0.43±0.01 | 0.48±0.03 | 0.72±0.05[b] | 1.28±0.04 | 2.04±0.08[c] | 2.55±0.01[c] |

Results are expressed as in Table 2.

C, standard diet; CO10, standard diet supplemented with 10% coconut oil; CO20, standard diet supplemented with 20% coconut oil.

[a,b,c] Significantly different from control: [a]$P<0.05$; [b]$P<0.005$; [c]$P<0.0005$.

Table 4.—Effect of 2% cholesterol feeding on cholesterol levels in neonatal chick liver Component (mg/g tissue)

| | | *Total cholesterol* | | *Free cholesterol* | | *Esterified cholesterol* | |
|---|---|---|---|---|---|---|---|
| | *Days of treatment* | *C* | *CHO* | *C* | *CHO* | *C* | *CHO* |
| Neonatal chick | 1 | 52.2±3.9 | 51.2±7.2 | 4.7±0.8 | 3.8±0.2 | 47.5±3.1 | 47.4±7.1 |
| | 4 | 14.9±1.4 | 14.5±2.5 | 3.1±0.3 | 2.9±0.2 | 11.8±1.2 | 11.6±2.1 |
| | 9 | 3.0±0.5 | 6.1±0.4[b] | 2.7±0.3 | 3.5±0.2 | 0.3±0.1 | 2.6±0.2[b] |
| | 15 | 2.9±0.1 | 7.9±1.5[a] | 2.5±0.1 | 3.6±0.4 | 0.4±0.1 | 4.3±1.2[a] |

Results are expressed as in Table 2.
C, standard diet; CHO, standard diet supplemented with 2% cholesterol.
[a,b] Significantly different from control: [a]$P<0.05$; [b]$P<0.005$.

were studied after 1-2 weeks of treatment. Table 8 shows that 10% coconut oil supplementation to the diet produced a significant increase in cholesterol content in LDL and HDL fractions in young chick, while in neonatal animals the increase was comparatively more pronounced in the VLDL fraction. Simultaneous supplementation of 10% coconut oil plus 1% cholesterol significantly increased total cholesterol content in IDL and, especially, in VLDL fractions (Table 9).

## DISCUSSION

Results presented in this paper corroborated the increase in plasma cholesterol when chicks were fed on a diet supplemented with cholesterol or saturated fat (coconut oil). However, the hypercholesterolemic effects of these lipids were clearly different according to the physiological conditions of the animals. Thus, young chicks were made hypercholesterolemic after a short cholesterol treatment (3 days), while neonatal chicks require 15 days of the same treatment to obtain a less significant hypercholesterolemia. This difference could be related to the high levels of plasma cholesterol found at hatching (6). As is well known, during embryonic development the liver and other tissues serve as a depository for preformed components derived from yolk resorption (10). Thus, the amount of hepatic cholesterol increased during the last embryonic period reaching at hatching a level about 10 times that found in 10-day-old embryo (4) and decreasing during the first week of neonatal life (5). Likewise, chick plasma cholesterol clearly decreased between 4 and 7 days after hatching, and remained practically constant from 7 days onwards (6).

The increase in chick plasma cholesterol observed after coconut oil supplementation to the diet corroborated previous results obtained in other animal species (11,12). It is important to note that the effect of coconut oil

Table 5.—Effect of coconut oil feeding on cholesterol levels in neonatal and young chick liver Component (mg/g tissue)

| | | *Total cholesterol* | | | *Free cholesterol* | | | *Esterified cholesterol* | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Days of treatment* | *C* | *CO10* | *CO20* | *C* | *CO10* | *CO20* | *C* | *CO10* | *CO20* |
| Neonatal chick | 7 | 7.63±0.27 | 7.77±0.26 | 7.61±0.25 | 4.18±0.15 | 4.05±0.11 | 3.81±0.21 | 3.45±0.23 | 3.72±0.25 | 3.80±0.44 |
| | 14 | 3.18±0.09 | 3.45±0.08 | 3.25±0.06 | 2.71±0.13 | 3.14±0.12 | 2.92±0.12 | 0.47±0.06 | 0.31±0.09 | 0.33±0.08 |
| Young chick | 7 | 4.14±0.11 | 3.91±0.12 | 4.00±0.36 | 3.11±0.10 | 3.24±0.08 | 3.28±0.43 | 1.03±0.15 | 0.67±0.15 | 0.72±0.04 |
| | 14 | 4.86±0.18 | 4.99±0.43 | 3.91±1.05 | 3.93±0.43 | 4.12±0.55 | 3.15±0.16 | 0.93±0.47 | 0.87±0.13 | 0.76±0.16 |

Results are expressed as in Table 2.

C, standard diet; CO10, standard diet supplemented with 10% coconut oil; CO20, standard diet supplemented with 20% coconut oil.

Table 6.—Effect of coconut oil or coconut oil plus cholesterol feeding on cholesterol levels in neonatal chick plasma *Component (mg/ml plasma)*

| | | *Total cholesterol* | | | *Free cholesterol* | | | *Esterified cholesterol* | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Days of treatment* | *C* | *CO10* | *CO10+CHO* | *C* | *CO10* | *CO10+CHO* | *C* | *CO10* | *CO10+CHO* |
| Neonatal chick | 7 | 1.67±0.08 | 2.03±0.05$^{a}$ | 5.29±0.01$^{c3}$ | 0.64±0.09 | 0.79±0.03 | 1.55±0.43$^{a}$ | 1.03±0.13 | 1.24±0.25 | 3.74±0.14$^{c3}$ |
| | 14 | 1.63±0.07 | 2.13±0.01$^{b}$ | 5.25±0.49$^{b2}$ | 0.48±0.01 | 0.58±0.05 | 1.56±0.37$^{a}$ | 1.15±0.06 | 1.55±0.17 | 1.69±0.18$^{c3}$ |
| | 21 | 1.62±0.04 | 1.86±0.01$^{b}$ | 4.84±0.82$^{a1}$ | 0.41±0.07 | 0.47±0.05 | 1.42±0.49 | 1.21±0.12 | 1.39±0.12 | 3.42±0.13$^{c3}$ |

Results are expressed as in Table 2.

C, standard diet; CO10, standard diet supplemented with 10% coconut oil; CO10+CHO, standard diet supplemented with 10% coconut oil plus 1% cholesterol.

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

$^{1,2,3}$ Significantly different from CO10: $^{1}P<0.05$; $^{2}P<0.005$; $^{3}P<0.0005$.

Table 7.—Effect of coconut oil or coconut oil plus cholesterol feeding on cholesterol levels in neonatal chick liver

| | | *Component (mg/g tissue)* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *Total cholesterol* | | | *Free cholesterol* | | | *Esterified cholesterol* | | |
| | *Days of treatment* | *C* | *CO10* | *CO10+CHO* | *C* | *CO10* | *CO10+CHO* | *C* | *CO10* | *CO10+CHO* |
| Neonatal chick | 7 | 7.94±0.76 | 9.00±0.72 | 13.48±0.61$^{b1}$ | 4.35±0.54 | 4.69±0.34 | 6.52±0.80 | 3.59±0.93 | 4.31±0.79 | 6.96±1.00$^{a1}$ |
| | 14 | 3.75±0.21 | 4.00±0.18 | 13.06±1.50$^{b2}$ | 3.27±0.21 | 3.77±0.04 | 6.09±0.98$^{a}$ | 0.48±0.09 | 0.21±0.11 | 6.98±1.79$^{b2}$ |
| | 21 | 3.50±0.12 | 4.25±0.30 | 17.50±1.05$^{c3}$ | 3.21±0.26 | 3.59±0.20 | 7.68±0.16$^{c3}$ | 0.29±0.09 | 0.65±0.27 | 9.82±1.06$^{c3}$ |

Results are expressed as Table 2.

C, standard diet; CO10, standard diet supplemented with 10% coconut oil; CO10+CHO, standard diet supplemented with 10% coconut oil plus 1% cholesterol.

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

$^{1,2,3}$ Significantly different from CO10: $^{1}P<0.05$; $^{2}P<0.005$; $^{3}P<0.0005$.

Table 8.—Effect of coconut oil feeding on cholesterol content in different lipoprotein fractions from neonatal and young chick

| | | | *Total cholesterol (mg/ml plasma)* | | |
|---|---|---|---|---|---|
| | | *Days of treatment* | *C* | *CO10* | *CO20* |
| Neonatal chick | HDL | 7 | 0.93±0.08 | 1.11±0.07 | 1.13±0.03 |
| | | 14 | 1.04±0.04 | 1.10±0.06 | 1.45±0.01[c] |
| | LDL | 7 | 0.36±0.03 | 0.53±0.01[a] | 0.66±0.01[c] |
| | | 14 | 0.42±0.01 | 0.62±0.01[a] | 1.30±0.04[c] |
| | IDL | 7 | 0.15±0.03 | 0.14±0.02 | 0.12±0.02 |
| | | 14 | 0.08±0.01 | 0.10±0.02 | 0.19±0.01[b] |
| | VLDL | 7 | 0.09±0.01 | 0.14±0.01[b] | 0.14±0.01[b] |
| | | 14 | 0.06±0.01 | 0.11±0.01[b] | 0.13±0.01[a] |
| Young chick | HDL | 7 | 0.85±0.01 | 1.07±0.02[b] | 0.94±0.19 |
| | | 14 | 0.87±0.01 | 1.23±0.01[c] | 1.41±0.04[c] |
| | LDL | 7 | 0.63±0.01 | 0.69±0.01[b] | 0.95±0.03[c] |
| | | 14 | 0.69±0.01 | 0.77±0.02[a] | 1.02±0.10[a] |
| | IDL | 7 | 0.07±0.02 | 0.08±0.01 | 0.08±0.03 |
| | | 14 | 0.06±0.01 | 0.05±0.01 | 0.16±0.03[a] |
| | VLDL | 7 | 0.04±0.01 | 0.06±0.01 | 0.10±0.01[b] |
| | | 14 | 0.05±0.01 | 0.05±0.01 | 0.10±0.01[b] |

Results are expressed as in Table 2.

C, standard diet; CO10, standard diet supplemented with 10% coconut oil; CO20, standard diet supplemented with 20% coconut oil.

[a,b,c] Significantly different from control: [a]P<0.05; [b]P<0.005; [c]P<0.0005.

was significant after 7 days of treatment in neonatal chick. These results suggest that neonatal chicks were more sensitive to saturated fat (coconut oil) than to cholesterol supplementation.

The hypercholesterolemic effect of cholesterol feeding was accompanied by an accumulation of cholesterol in the liver, interfering with the hepatic cholesterogenesis. Thus, addition of 2% cholesterol to the diet produced a clear inhibition of hepatic HMG-CoA reductase (13) and MVAPP decarboxylase (14) activities, as well as of mevalonate incorporation into nonsaponifiable lipids by liver slices (15) in neonatal chick. Similarly, cholesterol addition to the diet of young chick produced a rapid and significant increase in cholesterol content of hepatic microsomes (16), as well as a drastic decrease in the hepatic HMG-CoA reductase (16) and MVAPP decarboxylase (17) activities.

Table 9.—Effect of coconut oil or coconut oil plus cholesterol feeding on cholesterol conte in different lipoprotein fractions from neonatal and young chick

| | | | *Total cholesterol (mg/ml plasma)* | | |
|---|---|---|---|---|---|
| | | *Days of treatment* | *C* | *CO10* | *CO10+CH* |
| Neonatal chick | HDL | 7 | 1.02±0.08 | 1.18±0.09 | 1.11±0.1 |
| | | 14 | 1.07±0.08 | 1.16±0.10 | 1.33±0.1 |
| | | 21 | 0.90±0.04 | 1.09±0.08 | 1.55±0.5 |
| | LDL | 7 | 0.39±0.04 | 0.54±0.02[a] | 0.39±0.0 |
| | | 14 | 0.42±0.02 | 0.56±0.04[a] | 0.39±0.0 |
| | | 21 | 0.40±0.01 | 0.48±0.03 | 0.49±0.0 |
| | IDL | 7 | 0.18±0.03 | 0.17±0.03 | 0.26±0.0 |
| | | 14 | 0.08±0.01 | 0.11±0.02 | 0.32±0.06 |
| | | 21 | 0.07±0.02 | 0.06±0.01 | 0.32±0.07 |
| | VLDL | 7 | 0.09±0.01 | 0.15±0.02[a] | 0.48±0.06 |
| | | 14 | 0.06±0.01 | 0.07±0.01 | 0.57±0.05 |
| | | 21 | 0.03±0.01 | 0.04±0.01 | 0.40±0.05 |

Results are expressed as in Table 2.
C, standard diet; CO10, standard diet supplemented with 10% coconut oil; CO10+CHO standard diet supplemented with 10% coconut oil plus 1% cholesterol.
[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 CO10: [1]P<0.05; [2]P<0.005; [3]P<0.0005.

However, the hypercholesterolemic effect of coconut oil did not produce ar accumulation of cholesterol in chick liver, so that no inhibition of the hepatic cholesterogenic enzymes could be expected (18).

On the other hand, our results indicate an interactive influence of coconut oil and cholesterol when both constituents were supplemented simultaneously to the diet, in contrast with the different interactive influence observed in human (19,20). This discrepancy may be accounted for the different susceptibility to dietary cholesterol and for the quantity and form of added cholesterol. Our findings may be interpreted on the basis of the "cholesterol vehicle" function of this saturated fat, which may augment intestinal absorption of cholesterol and, therefore, its hypercholesterolemic effect (21).

Although the chick, like cebus monkey, is primarily an HDL animal with respect to cholesterol transport, the elevation of plasma lipid caused by saturated fat (coconut oil) feeding in neonatal chick is largely the result of increased LDL and VLDL pools, irrespective of the major circulation lipoprotein class (22). The mechanisms whereby certain saturated fatty acids raise LDL levels are not completely understood, although it has been suggested that this action is due to an impaired removal of LDL from the circulation and

enhanced synthesis of apo B containing lipoprotein (23). Likewise, coconut oil consumption may enhance the incorporation of saturated fatty acids into LDL receptor membrane and, ultimately, inhibits its function (24). The saturated fat-mediated increase in LDL flux could be also due to either an increase in VLDL production rates, increased conversion of VLDL to LDL, increased direct secretion of LDL by the liver, or various combination of these factors (25). Our results on the increase of both LDL- and VLDL-cholesterol by coconut oil feeding may suggest that the effects of dietary fatty acids on LDL are associated to the effects on VLDL production, as it has been recently reported in guinea pig (26).

On the other hand, our results obtained in neonatal chicks by simultaneous supplementation of coconut oil plus cholesterol are in agreement with those found in cockerel fed a cholesterol-supplemented diet (27,28). Recent studies by Hermier and Dillon (29) showed that VLDL fraction was enriched in cholesterol when young birds were fed a cholesterol-diet for 5 weeks. As in our experiemnts, 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. Likewise, the hypercholesterolemia of the newborn chick was mainly due to the accumulation of cholesterol-rich VLDL particles (6). All these results suggest an important role of this lipoprotein fraction in avian atherogenesis.

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