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Coconut oil induces short-term changes in lipid composition and enzyme activity of chick hepatic mitochondria

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We studied the short-term effects of a 20% coconut oil supplementation to the chick diet on lipid composition of liver and hepatic mitochondria, and changes that occurred in mitochondrial-associated enzymes as a result of this diet. No significant differences were observed in the lipid contents of liver when young chicks were fed the experimental diet, whereas hepatic mitochondria rapidly changed in response to this diet. Total cholesterol significantly increased in mitochondria at 24 hours of coconut oil diet feeding and decreased when dietary treatment was prolonged for 5 to 14 days. Changes in total mitochondrial phospholipids showed an inverse profile. A significant decrease in phosphatidylethanolamine and an increase in sphingomyelin were found at 24 hours. The cholesterol/phospholipid molar ratio significantly and rapidly (24 hours) increased in mitochondria from treated animals. Cytochrome oxidase activity drastically increased after 24 hours of experimental diet feeding and lowered to the control values when dietary manipulation was prolonged for 5 to 14 days. ATPase activity showed an inverse profile. Changes in cytochrome oxidase activity were parallel to changes in the cholesterol/phospholipid molar ratio, whereas changes in ATPase activity showed an inverse correlation with changes in this molar ratio. To our knowledge, this is one of the first reports on the very rapid response (24 hours) of mitochondrial lipid composition and function to saturated fat feeding. (J. Nutr. Biochem. 10:325–330, 1999)

Keywords: coconut oil; mitochondrial phospholipids; cholesterol; cytochrome oxidase; ATPase

Introduction

Several studies have demonstrated that dietary manipulations, especially lipid modifications, can alter the lipid composition of tissues and cells. Thus, changes in the lipid composition of experimental animals have been observed in mitochondrial and other membranes when the animals were fed diets supplemented with saturated or unsaturated fatty acids. The association between lipid and protein in biological membranes modulates many membrane-associated enzymes by changes in the physical properties of the surrounding membrane lipid.[1,2] The association between the different lipids also is involved in maintaining cell integrity and membrane fluidity.[3] Because phospholipids are by far the major lipid components of most membranes, alterations in these lipid species are of special interest because functional and pathologic consequences may be correlated.[4] Among the phospholipids, sphingomyelin (SM) stands out as the most reliable marker of their association with cholesterol.[5] The accumulation of SM in atherosclerotic lesions is well documented. Although 90% of cellular cholesterol and SM is located in the plasma membrane,[6] the remainder is distributed in other membrane fractions including endoplasmic reticulum and mitochondria.[7]

Few studies have been devoted to the influence of diet on phospholipid class distribution.[8,9] In addition, although many experiments have been designed to investigate the

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influence of relatively small amounts of different fatty acids, the quantity of fat in the diet has attracted less attention.[10–12] Different authors[13,14] have shown that long-term consumption of a diet enriched in saturated or unsaturated fatty acids can alter plasma lipid profiles. We recently reported that supplementation of 20% coconut oil to the chick diet for 14 days produced a clear damage to the hepatic mitochondria, as well as a rapid change in the fatty acid profiles in liver and hepatic mitochondria.[15]

The aim of the present study was to investigate the short-term effects of 20% coconut oil supplementation to the diet on the phospholipid class distribution in chick liver mitochondria. Lipid composition of liver also was determined. The chick has been recognized as a suitable animal model for studies on the comparative biochemistry of lipid metabolism because it is highly sensitive to dietary cholesterol.[16] We have shown that there is a strong increase in this sensitivity when cholesterol is administered simultaneously with coconut oil in the chick diet.[17] Under similar conditions, this heightened sensitivity is not observed in humans,[18] perhaps because of differences in susceptibility to dietary cholesterol between chicks and humans. Our recent results on young chick also showed 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.[19] Bearing in mind that the activities of some respiratory enzymes associated with the inner mitochondrial membrane are affected by nutritional modifications,[20,21] changes induced in cytochrome oxidase and ATPase activities also were studied.

Materials and methods

Newborn White Leghorn male chicks (*Gallus domesticus*) were obtained from a commercial hatchery and maintained in a chamber with controlled temperature (28°C) under a light cycle from 9:00 AM to 9:00 PM. Control animals were fed a commercial diet (Sanders A-00 Sanders, Granada, Spain) that contained (w/w) 42.0% carbohydrate (mainly starch), 6.6% fat, and 20.6% protein. The experimental diet was prepared by supplementation of 20% commercial cooking coconut oil to the standard diet and was used from the fourteenth day of chick life. This experimental diet was enriched in saturated fatty acids, especially lauric and myristic acids, which were nearly absent in the standard diet (*Table 1*). All animals had free access to water and food. Experiments were carried out after 1, 5, and 14 days of dietary manipulation.

Chicks were sacrificed by decapitation 6 hours after free access to water and food. Livers were rapidly removed, minced, and pooled. A 3-g sample of each pool was placed in a beaker containing approximately 8 vol of 0.25 M sucrose and homogenized with a motor-driven all-glass Potter-Elvehjem homogenizer. The isolation of mitochondria was performed as described by Sánchez-Amate et al.[22] Briefly, after centrifugation of homogenate at 400 g for 10 minutes, the supernatant was recentrifuged at 800 g for 10 minutes. The resulting supernatant fraction was centrifuged at 5,000 g for 10 minutes to obtain the mitochondrial pellet, which was washed with 0.25 M sucrose, resuspended by homogenation, and recovered after a second centrifugation at 5,000 g. All centrifugations were performed at 4°C, and samples were kept on ice between centrifugations. Purity of mitochondrial preparations (approximately 95%) was measured by determining different mitochondrial and microsomal marker enzyme activities.[23]

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

Fatty acid	Control	+20% Coconut oil
8:0	ND	2.18 ± 0.05[a]
10:0	ND	3.72 ± 0.08[a]
12:0	ND	37.29 ± 0.28[a]
14:0	0.77 ± 0.02	15.11 ± 0.22[a]
16:0	22.36 ± 0.05	13.02 ± 0.12[a]
16:1 n-7	3.27 ± 0.01	0.82 ± 0.01[a]
18:0	8.56 ± 0.02	4.78 ± 0.08[a]
18:1 n-9	32.43 ± 0.05	13.65 ± 0.06[a]
18:2 n-6	24.60 ± 0.09	7.42 ± 0.04[a]
18:3 n-3	0.85 ± 0.01	0.21 ± 0.01[a]
20:2 n-6	2.53 ± 0.02	0.63 ± 0.01[a]
20:3 n-6	1.09 ± 0.03	0.27 ± 0.01[b]
20:4 n-6	1.56 ± 0.02	0.39 ± 0.01[a]
22:5 n-3	1.70 ± 0.03	0.42 ± 0.02[a]
ΣSaturated	31.69 ± 0.09	76.10 ± 0.40[a]
ΣUnsaturated	68.03 ± 0.10	23.81 ± 0.08[a]
ΣMUFA	35.70 ± 0.06	14.47 ± 0.06[a]
ΣPUFA	32.33 ± 0.08	9.34 ± 0.02[a]
Σn-3	2.55 ± 0.06	0.63 ± 0.02[a]
Σn-6	29.78 ± 0.28	8.71 ± 0.04[a]
ΣSat./ΣUnsat.	0.46 ± 0.06	3.20 ± 0.02[a]
ΣSat./ΣPUFA	0.98 ± 0.01	8.15 ± 0.05[a]

Note: Results are expressed as means ± SEM of three determinations. ND–not detected. Statistical significance is indicated by [a]$P < 0.0001$ or [b]$P < 0.005$ (Student's *t*-test).
MUFA–monounsaturated fatty acids. PUFA–polyunsaturated fatty acids.

Total lipids from liver and mitochondria were extracted with chloroform/methanol 2:1 (v/v) as described by Folch et al.[24] Cholesterol and triacylglycerol contents were determined by using specific commercial kits from Boehringer Mannheim (Mannheim, Germany). Phospholipids were measured by the method of Bartlett.[25] The separation of phospholipid classes was achieved by thin layer chromatography according to the method of Higgins.[26]

ATPase activity was measured as described by Pullman et al.[27] and cytochrome oxidase activity as described by Sottocassa et al.[28]

Results are expressed as mean values ± SEM of three experiments carried out with pools of six animals. Each experimental value was mean of triplicate determinations. Student's *t*-test was used to compare the values obtained from control and treated animals.

Results and discussion

In spite of the different fat contents of control and experimental diets, no interference in the growth rate of young chicks was found. No significant differences were observed in body and liver weight gains among groups fed different diets. No differences were observed in the amount of diet consumed by chicks fed different diets. Previous results showed that the addition of 20% coconut oil to the diet produced an accumulation of liver glycogen, but no influence derived from protein deficiency has been observed.[15]

Chemical composition of liver and hepatic mitochondria from animals fed the standard diet remained practically constant during the assayed period. Because of this, only one value was considered as control for each constituent. *Table 2* shows that no significant differences were observed in the lipid contents of liver when 14-day-old chicks were

Table 2 Short-term effects of 20% (w/w) coconut oil supplementation to the diet on lipid composition of young chick liver

Component	Control	1 Day	5 Days	14 Days
Total cholesterol	7.39 ± 0.05	8.58 ± 0.90	8.60 ± 1.91	7.46 ± 1.53
Triglycerides	8.74 ± 0.19	10.60 ± 2.00	7.55 ± 1.56	6.48 ± 1.28
Phospholipid	34.12 ± 2.10	28.32 ± 2.10	28.12 ± 2.02	30.08 ± 3.01

Note: Results (μmol/g tissue) are expressed as means ± SEM of three experiments carried out with pools of six animals. Triplicate determinations were made in each experiment.

fed a diet supplemented with 20% coconut oil, in contrast to the rapid and significant hypercholesterolemia found after only 24 hours of this dietary manipulation.[19]

Lipid composition of hepatic mitochondria changed rapidly in response to the supplemented diet. As can be seen in *Table 3,* total cholesterol increased following 24 hours of coconut oil feeding. When this dietary treatment was prolonged for 5 days, levels of mitochondrial cholesterol decreased. After 14 days of feeding, no significant differences were found with respect to the control value of mitochondrial cholesterol. Changes in total phospholipids showed a different profile: A clear decrease was observed at 24 hours, with a clear tendency to increase when this diet was continued for 5 to 14 days. Analysis of phospholipid class distribution in control and treated groups shows that phosphatidylcholine (PC) and phosphatidylethanolamine (PE) levels significantly decreased as a consequence of 24 hours of coconut oil feeding, whereas SM significantly increased. In any case, these levels tended to reach the control values at the end of the assayed treatment. However, no significant differences were found in the phosphatidylserine (PS) and phosphatidylinositol (PI) levels. As a consequence of these changes, the PE/PC, PC/SM, and PE/SM ratios decreased significantly after treatment for 24 hours.

It is known that the phospholipid composition of a particular membrane entity is a major determinant of the level of cholesterol associated with that membrane. Thus, SM correlates closely with the amount of cholesterol in different membranes.[29] The presence of SM influences cholesterol movement[30] and distribution[31] among cellular and/or subcellular membranes. It has been suggested that SM may participate in the maintenance of cell cholesterol homeostasis by regulating the uptake and intracellular processing of low density lipoproteins.[32] The influence of SM on the structure and function of reconstituted high density lipoproteins also has been reported.[33] Likewise, the metabolism of plasma cholesterol could be influenced by the differences in the molecular species of PC included in lipoproteins.[34] On the contrary, several reports have provided evidence suggesting that the hypocholesterolemic action of some dietary amino acids, as well as eritadenine and other adenosine analogues,[35–38] might be associated with the alteration of hepatic phospholipid metabolism. The PE/PC ratio in hepatic microsomes exhibited a significant inverse correlation with plasma cholesterol concentrations.[38]

Our results in chick liver mitochondria are in agreement with all of the above findings, demonstrating the relationship between changes induced by dietary treatments in cholesterol and different phospholipid classes.

On the other hand, most studies have been devoted to the effects on mitochondrial lipid composition and function when normal diets are supplemented for an extended time with different fatty acids. Although there are some exceptions,[9] the quantitative distribution of phospholipid classes in a given membrane is not altered significantly by dietary fatty acid supplementation for prolonged periods. It has been suggested that a homeostatic mechanism may buffer the membrane from the effects of changes in the nature of the dietary lipid intake[12,39] in order to preserve a relatively constant level of membrane fluidity.[40] Our previous results showed that supplementation of 20% coconut oil to the chick diet produced rapid (24 hours) changes in the fatty

Table 3 Short-term effects of 20% (w/w) coconut oil supplementation to the diet on lipid composition of chick liver mitochondria

Component	Control	1 Day	5 Days	14 Days
TC	0.057 ± 0.005	0.079 ± 0.002[a]	0.048 ± 0.009	0.071 ± 0.009
PL	0.199 ± 0.021	0.158 ± 0.027	0.256 ± 0.053	0.213 ± 0.001
PC	0.075 ± 0.005	0.054 ± 0.003[a]	0.092 ± 0.022	0.075 ± 0.008
PE	0.056 ± 0.007	0.024 ± 0.002[a]	0.062 ± 0.015	0.059 ± 0.005
PS	0.041 ± 0.012	0.046 ± 0.003	0.056 ± 0.014	0.045 ± 0.003
SM	0.014 ± 0.003	0.026 ± 0.002[a]	0.026 ± 0.007	0.018 ± 0.001
PI	0.013 ± 0.004	0.012 ± 0.002	0.020 ± 0.005	0.016 ± 0.002
PE/PC	0.75 ± 0.09	0.44 ± 0.04[a]	0.67 ± 0.22	0.78 ± 0.10
PC/SM	5.36 ± 0.68	2.08 ± 0.29[a]	3.54 ± 0.89	4.17 ± 0.42
PE/SM	3.79 ± 0.95	0.92 ± 0.11[a]	2.38 ± 0.86	3.33 ± 0.33

Note: Results (μmol/mg protein) are expressed as means ± SEM of three experiments carried out with pools of six animals. Triplicate determinations were made in each experiment. Statistical significance is indicated by [a]$P < 0.05$ with respect to the control value.
TC–total cholesterol. PL–phospholipid. PC–phosphatidylcholine. PE–phosphatidylethanolamine. PS–phosphatidylserine. SM–sphingomyelin. PI–phosphatidylinositol.

Table 4 Short-term effects of 20% (w/w) coconut oil supplementation to the diet on cholesterol/different phospholipids molar ratios in chick liver mitochondria

	Control	1 Day	5 Days	14 Days
C/P	0.29 ± 0.05	0.50 ± 0.02[a]	0.19 ± 0.04	0.33 ± 0.04
C/PC	0.76 ± 0.13	1.46 ± 0.09[a]	0.52 ± 0.16	0.94 ± 0.15
C/PE	1.02 ± 0.27	3.29 ± 0.22[a]	0.77 ± 0.24	1.20 ± 0.19
C/PS	1.39 ± 0.33	1.71 ± 0.12	0.86 ± 0.21	1.57 ± 0.23
C/SM	4.07 ± 0.77	3.04 ± 0.40	1.85 ± 0.63	3.94 ± 0.60
C/PI	4.38 ± 0.76	6.58 ± 0.94	2.40 ± 0.76	4.43 ± 0.72

Note: Results are expressed as means ± SEM of three experiments carried out with pools of six animals. Triplicate determinations were made in each experiment. Statistical significance is indicated by $^{a}P < 0.05$ with respect to the control value.
C–cholesterol. P–phospholipid. PC–phosphatidylcholine. PE–phosphatidylethanolamine. PS–phosphatidylserine. SM–sphingomyelin. PI–phosphatidylinositol.

acid profile of liver and hepatic mitochondria.[15] However, most of the mitochondrial parameters measured recuperated the control values when this diet was supplied for 5 to 14 days. Mitochondrial ratios of saturated/unsaturated and saturated/polyunsaturated fatty acids also showed a rapid (24 hours) increase in response to the experimental diet feeding.

Cholesterol plays a key role in mitochondrial membranes because it appears to maintain the bilayer matrix in an intermediate fluid state by regulating the mobility of phospholipid fatty acyl chains.[41] An increase in cholesterol with relation to phospholipid has been shown to decrease fluidity in both biological and artificial membranes.[42,43] As can be seen in *Table 4*, the cholesterol/phospholipid (C/P) molar ratio significantly and rapidly (24 hours) increased in mitochondria from animals fed our experimental diet. The ratio of cholesterol to the main phospholipids (C/PC and C/PE) also changed with the same profile. These results suggest the induction of a clear decrease in mitochondria fluidity caused by coconut oil supplementation to the diet. We recently showed that changes in the C/P molar ratio induced by saturated fat feeding are related to changes in the lipidic order parameter from the steady-state fluorescence an isotropy of 1,6-diphenyl-1,3,5-hexatriene labelled chick lipoprotein.[44] Our results are also in agreement with those previously reported[15] regarding changes in mitochondrial fatty acid composition, which showed that the increase in the degree of fatty acid saturation reduced the fluidity of the liver phospholipids.[45] However, all of these mitochondrial parameters decreased to the control values when 20% saturated fat was supplemented to the diet for 5 to 14 days.

Different data have suggested that some mitochondrial enzyme activities can be altered by long-term treatments with different dietary fats. However, few studies have looked specifically at the effects of dietary manipulations over the short term. In the present experiment, we studied the possible influence of rapid changes induced by coconut oil supplementation to the diet on cytochrome oxidase and ATPase mitochondrial activities. Data in *Table 5* show that cytochrome oxidase activity drastically increased after 24 hours of experimental diet feeding. When dietary manipulation was prolonged for 5 to 14 days, levels of this activity decreased, reaching values similar to those of controls. ATPase activity showed an inverse profile, decreasing at 24 hours and increasing thereafter. It is important to remark that these changes were very rapid, occurring only 24 hours after supplementation to the standard diet with 20% coconut oil. Changes in cytochrome oxidase exhibited a positive correlation with those in the C/P, C/PC, and C/PE ratios, whereas ATPase activity showed an inverse correlation with these ratios.

Similarly, heart mitochondria isolated from rats fed a lipid-free diet supplemented with sardine oil for 30 days showed significantly lower rates of phosphorylating respiration and cytochrome oxidase activity.[21] Similar results were obtained when dogs were fed a diet supplemented with menhaden oil for 5 to 26 weeks.[46] These findings are in contrast to the simultaneous elevation of mitochondrial ATPase.[21] It has been suggested that ATPase activity could be related to mitochondrial fatty acid composition. Thus, this activity decreased or increased as a function of the ratio of n-6 to n-3 fatty acids.[20] Our previous results showed a significant decrease in the percentage of both linoleic and arachidonic acids in chick liver and mitochondria after 24 hours of coconut oil supplementation to the diet, but these percentages reached the control values when dietary manipulation was prolonged for 14 days.[15] Likewise, mitochondrial saturated/unsaturated and saturated/polyunsaturated

Table 5 Short-term effects of 20% (w/w) coconut oil supplementation to the diet on chick mitochondrial-associated enzymes

Specific activity (μmol/min.mg protein)	Control	1 Day	5 Days	14 Days
Cytochrome oxidase	0.23 ± 0.02	0.40 ± 0.04[a]	0.27 ± 0.02	0.25 ± 0.04
ATPase	7.10 ± 0.49	5.16 ± 0.04[a]	7.67 ± 0.68	6.97 ± 0.87

Note: Results are expressed as means ± SEM of three experiments carried out with pools of six animals. Triplicate determinations were made in each experiment. Statistical significance is indicated by $^{a}P < 0.05$ with respect to the control value.

ratios increased at 24 hours but reached the level of control values after 14 days of the same treatment.

In conclusion, the results of the present study suggest that the rapid response of cytochrome oxidase and ATPase activities to coconut oil supplementation to the diet could be related to changes in the mitochondrial C/P molar ratio and to changes in mitochondrial fatty acid composition.

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