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Dietary fat saturation produces lipid modifications in peritoneal macrophages of mouse

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Abstract

We investigated the effects of a saturated fat diet on mice lipid metabolism in resident peritoneal macrophages. Male C57BL/6 mice were weaned at 21 days of age and assigned to either the experimental diet, containing coconut oil (COCO diet), or the control diet, containing soybean oil as fat source. Fat content of each diet was 15% (w/w). Mice were fed for 6 weeks until sacrifice. In plasma of mice fed the COCO diet, the concentration of triglyceride, total cholesterol, HLD- and (LDL+VLDL)-cholesterol, and thiobarbituric acid-reactive substances (TBARS) increased, without changes in phospholipid concentration, compared with the controls. In macrophages of COCO-fed mice, the concentration of total (TC), free and esterified cholesterol, triglyceride, phospholipid (P) and TBARS increased, while the TC/P ratio did not change. The phospholipid compositions showed an increase of phosphatidylcholine and phosphatidylserine + phosphadytilinositol, a decrease of phosphatidylethanolamine, and no change in phosphatidylglycerol. ^3H_2O incorporation into triglyceride and phospholipid fractions of macrophages increased, while its incorporation into free cholesterol decreased. Incorporation of [^{3}H]cholesterol into macrophages of COCO-fed mice and the fraction of [^{3}H]cholesterol ester increased. COCO diet produced an increase in myrystic, palmitic and palmitoleic acids proportion, a decrease in linoleic and arachidonic acids and no changes in stearic and oleic acids, compared with the control. Also, a higher relative percentage of saturated fatty acid and a decrease in unsaturation index ($p < 0.001$) were observed in macrophages of COCO-fed mice. These results indicate that the COCO-diet, high in saturated fatty acids, alters the lipid metabolism and fatty acid composition of macrophages and produces a significant degree of oxidative stress.

Keywords: Peritoneal macrophages; Coconut oil; Soybean oil; Fatty acids; Lipids; Thiobarbituric acid-reactive substance (TBARS)

1. Introduction

Macrophages are immune, inflammatory cells characterized by an active energetic, oxidative and lipid metabolism. Several functions of macrophages depend on their lipid composition, such as signal reception and transduction, phagocytosis, and synthesis of oxidized-lipid and other inflammatory mediators (e.g. eicosanoids and platelet activator factor) [1]. The phospholipids of murine peritoneal macrophages are highly enriched with arachidonic acid, which acts as second messenger and as substrate for inflammatory mediator synthesis [2]. Macrophages play an important role in the processing of cholesterol derived from metabolism of plasma lipoproteins as well as in the reutilization of cholesterol from damaged or senescent cells [3,4]. Monocyte-derived macrophages are especially important in the processing of cholesterol in atherosclerotic lesions. There is a well-documented association between the intake of saturated fat in the diet, elevated plasma LDL cholesterol levels, and increased risk of cardiovascular disease [5].

Membrane lipid modification can be achieved in vivo by dietary means or in vitro by controlling the lipid environment of the cells [6,7]. It has been shown that the composition and distribution of dietary saturated fatty acids determine in part the composition and distribution of fatty acids in liver and adipose tissue, as well as in fasting plasma triacylglyceroles of young piglets. The amount, not the position, of dietary 16:0 of coconut oil and palm oil determines the 16:0 content in piglet adipose tissue [8]. Fatty acids play a role in modulating cellular metabolism. Indeed, fatty acid-rich diets (with 20% by weight of hydrogenated coconut oil or unsaturated olive, safflower, or menhaden oil)

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can lead to changes in phagocytosis, enzyme activity (hexokinase, citrate synthase, glucose-6-phosphate dehydrogenase, and glutaminase), and production of cytokines (interleukin-1 and -6, tumor necrosis factor-α), eicosanoids (prostaglandins PGE_2 and 6-keto-$PGF_{1\alpha}$), and reactive species (superoxide, hydrogen peroxide, and nitric oxide) in resident and TG-elicited macrophages [6,9-11].

Lipid peroxidation is a free radical reaction whereby polyunsaturated fatty acids and phospholipids undergo degradation by a chain reaction that results in the formation of lipoperoxides, various aldehydes (eg, malondialdehyde), and short chain hydrocarbons (eg, ethane, propane, pentane), among others [12]. Immune cells are particularly sensitive to oxidative stress because of the high percent of polyunsaturated fatty acids in their plasma membranes and a higher production of reactive oxygen species, which is part of their normal function [12]. Dietary lipid plays a key role in determining cellular susceptibility to oxidative stress. It has been concluded that changes in fat level and fatty acid composition of the diet alters the mucosal cell membrane lipid composition in the rat large intestine and influences susceptibility of mucosal cell lipid to peroxidation [13]. Oxidative stress induced by dietary saturated fatty acids could alter cellular membranes, modifying the lipid composition and function of peritoneal macrophages [11,14].

Knowing that mouse peritoneal macrophages are commonly used as a model system for elucidating the mechanisms that regulate human macrophage metabolism and function, the aim of this study was to determine the effects of dietary saturated fat on the lipid metabolism and fatty acid composition of resident peritoneal macrophages.

2. Methods and Materials

2.1. Chemical, media and reagents

Dulbecco's modified Eagle's medium (DMEM) phenol red-free and fetal calf serum (FCS) were purchased from Gibco BRL. [^{3}H]-H_2O (3.70 GBq/g) and [1,2-^{3}H(N)]-cholesterol (9.25 MBq/g) were purchased from Dupont, New England Company (Boston, MA, U.S.A). Antibiotics and lipid standards were acquired from Sigma Chemical Co (St. Louis, MO). The plasma concentrations of glucose, total cholesterol, HDL-cholesterol (HDLc), phospholipids and triglyceride were determined by enzymatic method using kits from Boehringer Mannheim Diagnostics (Indianapolis, IN, U.S.A). All other chemicals were of reagent grade and were purchased from Merck Laboratory (Buenos Aires, Argentina).

2.2. Animals and diets

Adult male C57BL/6 mice bred in our animal facilities and maintained in a humidity and temperature (21-23°C) controlled environment with a 12-h light:dark cycle were used. The mice came from dams which were maintained on standard lab chow and were weaned at 21 days of age and immediately assigned randomly to either the experimental diet, containing coconut oil (COCO diet), or the control diet, containing soybean oil as fat source, respectively. Mice were housed in individual stainless steel cages and given free access to food and water throughout the entire 6 weeks of the experimental period. Diets were isocaloric and contained identical compositions except for the fat type. The fat content was 15% (w/w) in both the control and COCO diets. The fatty acid compositions of diets are shown in Table 1. Coconut oil also contains 8% caprylic (8:0) and 6% capric (10:0) acids [15], but these were not detected under our working conditions. Diets were prepared according to AIN-93 for laboratory rodents [16].

Table 1
Fatty acid composition (wt %) of the experimental diets[1]

Fatty Acid[2]	Dietary fat source	
	Soybean oil	Coconut oil[3]
12:0	0.12	41.6
14:0	trace[3]	16.8
16:0	9.0	10.9
16:1 (n-7)	0.2	—
18:0	5.8	13.6
18:1 (n-9)	22.6	2.7
18:2 (n-6)	52.4	2.1
18:3 (n-3)	8.2	0.2

[1] The difference between the sum of the percentages and 100 represents unidentified fatty acids.

[2] Number of carbon atoms:number of double bonds, followed by the position of the first double bond relative to the methyl end (n-) of the fatty acid.

[3] ≤0.1% (trace). Fatty acids shorter than 12:0 were not detected.

The oils were stored in opaque containers at -70°C and the diets were prepared fresh daily by addition of the appropriate amount of oil to a mix of the other dietary components. Body weight and food intake were recorded daily.

2.3. Plasma determinations

Between 8.00-9.00 a.m. of the last experimental day mice were killed by decapitation. Blood was collected in EDTA-coated tubes (150 mg EDTA/L in 9 g NaCl/L), and plasma was prepared by centrifugation. Plasma glucose, triglyceride, phospholipids, total cholesterol and HDL-cholesterol concentrations were measured by enzymatic methods (kits from Boehringer) using fresh plasma from mice that had been deprived of food for 12 h. The difference between total cholesterol and HDL cholesterol was assumed to be the cholesterol associated with (LDL + VLDL).

2.4. Isolation and preparation of peritoneal macrophages

Resident peritoneal macrophages were prepared in a LPS-free system as described previously [17]. Peritoneal

cells were prepared by washing the peritoneal cavity with ice-cold Hank's balanced saline solution, Ca^{2+}-, Mg^{2+}- and phenol red-free ($HBSS^-$) containing 1 mM EDTA and 1% BSA. After washing the peritoneal cells twice with cold $HBSS^-$, they were suspended in DMEM and 2 x 10^6 cells were placed in each well of 12-well culture plates (Corning Glass Works, Corning, NY, USA). Macrophages were allowed to adhere for 2 h at 37°C in a 95% air-5% CO_2 atmosphere. The dishes were then thoroughly washed three times with ice-cold $HBSS^-$ to remove non-adherent cells.

Macrophages were further cultured 24 h in phenol red-free DMEM supplemented with 10% FCS, 50 μg/ml gentamicin, 50 μg/ml penicillin, and 50 μg/ml fungizone.

2.5. Peritoneal macrophage suspension

Adherent cells were harvested by incubation for 1 h at 4°C with 1 ml of cold 5 mM EDTA/5% FCS in $HBSS^-$. The cell suspension was aspirated and centrifuged (200 x g, 10 min, 4°C). Cells were washed with cold $HBSS^-$. 250 μl of cell suspension (4 x 10^6 cell/ml of $HBSS^-$) were treated with 250 μl of lysis solution (20 mM Tris-succinate pH 7.8 containing protease inhibitor cocktail and 0.1% Triton X-100).

2.6. Cell viability

After being harvested and rinsed with cold $HBSS^-$ the adherent cells were centrifuged. The pellet was resuspended in 1 ml of $HBSS^-$. The cell suspension (10 μl) was mixed with the same volume of 0.1% Trypan blue in PBS. After 3 min incubation on ice, the cells were screened for viability by visible microscopy. The viability of these cells as evaluated by Trypan blue exclusion was never below 95%.

2.7. Lipid determinations

One ml of macrophage suspension containing 4x10^6 cells was centrifuged and lipids from the cellular pellet were extracted with chloroform:methanol (2:1) according to the method of Folch et al. [18]. An aliquot of the lipid extracts was taken to determine total cholesterol, and another one to separate the different lipid fractions by thin-layer chromatography (TLC) using plates coated with silica gel G (Merck, Darmstadt, Germany), with an *n*-hexane:diethyl ether:acetic acid (80:20:1, v/v/v) solvent system. Lipids were detected by exposing the plates to iodine vapors. After eluting the scraped bands, aliquots were used for mass determination according to the methods of Rausser et al. [19] for phospholipids, Sardesai et al. [20] for triglycerides, and of Zak et al. [21] after saponification [22] for free and esterified cholesterol. On average, 90% of cholesterol mass was recovered from thin-layer chromatography. Phospholipids were separated into component species by TLC using silica gel G plates and chloroform: methanol: water (65:25:4 v/v) as solvent system. The individual phospholipids were identified, recovered and quantified for phosphorus content as indicated above. The results were expressed as percentage of total phospholipid phosphorus content. The position of neutral lipids and individual phospholipids was determined using the respective standards.

2.8. Incorporation of ^3H from H_2O into lipids of peritoneal macrophages

After 24 h of culture the peritoneal macrophages (2 x 10^6 cells) were incubated with 0.1 μCi of [^{3}H]-H_2O for 60 min at 37°C in a 95% air-5% CO_2 atmosphere. After that, the medium was aspirated and the cells were rinsed with saline solution until no radioactivity was detected in the wash solution. Lipid extract was obtained by the Folch procedure, and lipid fractions were separated by TLC plates (see Lipid determinations). Bands were detected using iodine vapors, scraped off and their radioactivity quantified in a Beckman LS 100 C Liquid Scintillation Counter. The results are expressed as μmol ^{3}H incorporated/h/mg protein.

2.9. Incorporation of [^3H] cholesterol by peritoneal macrophages

After 24 h of culture the peritoneal macrophages (2 x 10^6 cells) were incubated with 0.125 μCi of [^{3}H]-cholesterol for 60 min at 37°C in a 95% air-5% CO_2 atmosphere. Lipid extract, separation of lipid fractions by TLC and radioactivity quantification of cholesterol and cholesterol ester bands were done as indicated above. The results are expressed as pmol of ^{3}H incorporated/h/mg protein.

2.10. Determination of lipid peroxidation (cell TBARS)

Determination of thiobarbituric acid reactive substances (TBARS) was used as a measure of lipid peroxidation. A volume of 1 ml of cell suspension containing 1 x 10^6 cells was mixed with 1 ml 0.25 M HCl containing 3.75% TBA and 15% trichloroacetic acid, and boiled for 15 min. After centrifugation at 1500 x g for 15 min the absorbance was read at 532 nm. The results, expressed as nmol malondialdehyde (MDA)/10^6 cells, were obtained from standard using an MDA stock solution prepared by hydrolysis of tetramethoxypropane [23].

2.11. Fatty acid composition of peritoneal macrophages

One ml of macrophage suspension was saponified by treatment with 10% (w/v) KOH in ethanol plus 500μl of methanol in order to facilitate the subsequent extraction step. The free fatty acids were recovered after acidification with 0.7 N HCl and extracted twice with petroleum ether (bp 30-40). Free fatty acids were esterified 1h at 64°C with boron trifluoride solution (20% in methanol). The methyl esters were extracted with hexane, dried down under nitrogen and stored at -70°C until gas-liquid-chromatography

analysis. The GLC conditions included the following: Varian 3300 Chromatograph (Barckeley, CA) equipped with a 5848-A Chromosorb WAW-DMCS (100-200 mesh) (Supelco, Inc, Bellefonte PA, USA). The oven temperature was programmed to rise from 140 to 220°C at a rate of 3°C per min after 1-min initial holding interval. Authentic standards of fatty acid methyl ester mixture were used to identify and quantify macrophages fatty acid composition. Fatty acids methyl esters were identified comparing their relative retention times in presence of eicosa-11-monoenoic acid as internal standard [24].

2.12. Protein determination

Protein was measured by the method of Wang and Smith [25] for cell homogenate containing detergents, using bovine serum albumin (BSA) fraction V as standard.

2.13. Statistical analysis

Data are presented as mean ± SEM. They were tested by Student's test for non correlated data. Statistical significance was accepted at $p < 0.05$.

3. Results

3.1. Growth, food intake and plasma concentrations of glucose, protein, lipids and TBARS

The initial body weight (g) of the mice in both dietary groups was the same (9 ± 0.7). At the time of killing, there was no significant effect of the saturated fatty acid dietary manipulation on the body weight gain of the mice. As the mice grew up, the daily food consumption (g) increased similarly in both dietary groups (Table 2). No effect of dietary treatment was observed on plasma glucose level. The COCO-fed mice had significantly higher plasma triglyceride and cholesterol concentrations than the control-fed mice. The high plasma cholesterol observed in the COCO-fed group was related to the increased HDL and VLDL+ LDL cholesterol fractions. There were no differences in plasma phospholipid concentrations between the dietary groups. The level of plasma TBARS increased in the COCO-fed group compared with that of the control.

3.2. Effect of the diets on the macrophage lipid compositions and TBARS content

As shown in Table 3, the concentration of total cholesterol (which was related to the increased free and esterified cholesterol fractions), triglyceride and phospholipids increased in the peritoneal macrophages of COCO-fed mice in relation to those of the control group. Consequently, the ratios of free cholesterol/esterified cholesterol and total cholesterol/phospholipids were not changed by the COCO diet. The cell protein concentration was not affected by the diet. In addition to the changes in lipid composition of macrophages of mice fed on the COCO diet we also observed an increment of cell TBARS levels in relation to those of mice fed on the control diet.

Table 2
Effect of the diet on body weight gain, food intake and plasma glucose, lipids and thiobarbituric acid reactive substances[1]

	Control	COCO	
Initial body wt (g)	9.11 ± 0.94	9.03 ± 0.58	NS
Body wt gain, (g/6 weeks)	14.00 ± 0.38	13.05 ± 0.32	NS
Food intake, (g/day)			
1st week	5.40 ± 0.30	5.90 ± 0.42	NS
2nd week	7.58 ± 0.42	7.15 ± 0.45	NS
3rd week	8.65 ± 0.24	8.76 ± 0.37	NS
4th week	10.18 ± 0.42	10.44 ± 0.22	NS
5th week	10.40 ± 0.30	10.61 ± 0.18	NS
6th week	11.40 ± 0.30	11.51 ± 0.58	NS
Plasma glucose (g/l)	0.82 ± 0.02	0.79 ± 0.04	NS
Plasma lipids, (g/l)			
Triglyceride	1.16 ± 0.06	1.97 ± 0.09	$p < 0.001$
Total cholesterol	0.72 ± 0.02	1.17 ± 0.03	$p < 0.001$
HDL cholesterol	0.44 ± 0.07	0.67 ± 0.05	$p < 0.01$
LDL + VLDL cholesterol	0.28 ± 0.06	0.50 ± 0.04	$p < 0.01$
Phospholipids (Pi)	0.08 ± 0.007	0.09 ± 0.008	NS
TBARS, (nmol MDA/ml)	7.12 ± 0.96	11.75 ± 1.34	$p < 0.01$

[1] Values are means ±SEM for 10 mice/group. Means differences were determined by using Student's test. The *P* values in the table indicate significant differences between feeding diet containing coconut oil (COCO) and soybean oil (control group). NS, not significant.

Table 3
Effect of the diet on peritoneal macrophages lipid compositions and thiobarbituric acid reactive substances[1]

	Control	COCO	
(μg/mg protein)			
Total Cholesterol (TC)	96.12 ± 1.88	125.33 ± 3.34	$p < 0.01$
Free Cholesterol (FC)	83.52 ± 1.97	112.89 ± 2.83	$p < 0.001$
Esterified Cholesterol (EC)	10.30 ± 1.02	15.28 ± 0.69	$p < 0.01$
Triglyceride	58.04 ± 2.03	73.35 ± 2.18	$p < 0.001$
Phospholipids	218.83 ± 12.37	285.60 ± 13.2	$p < 0.01$
FC/EC	8.93 ± 0.35	7.42 ± 0.31	NS
TC/Phospholipids	0.44 ± 0.07	0.43 ± 0.08	NS
Protein (mg/10^7 cells)	1.16 ± 0.12	1.03 ± 0.25	NS
TBARS (nmol MDA/mg protein)	5.84 ± 0.41	11.29 ± 0.75	$p < 0.001$

[1] Values are means ± SEM for four experiments with three COCO- and control-fed mice each. Chemical determinations of each animal were made in two separate cell preparations. Means differences were determined by using Student's test. The *P* values in the table indicate significant differences between feeding diet containing coconut oil (COCO) and soybean oil (control group). NS, not significant.

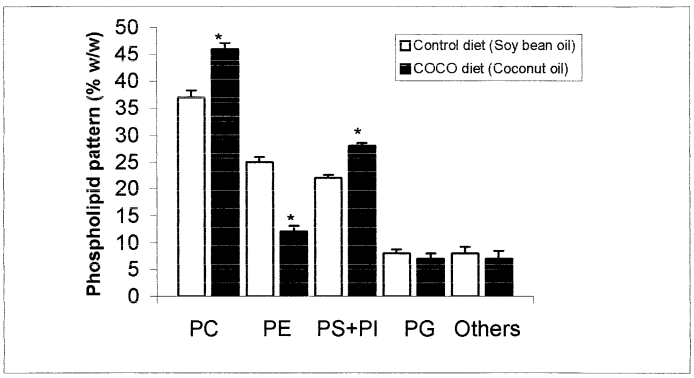

Fig. 1. Phospholipid pattern in mouse peritoneal macrophages. PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS + PI, phosphatidylserine plus phosphatidylinositol; PG, phosphatidylglycerol. The graph represents the means ± SEM from three experiments with three COCO- and control-fed mice each. Chemical determinations of each animal were made in two separate cell preparations. *indicates significant differences ($p < 0.05$) between macrophages from mice fed the COCO diet and those fed the control diet.

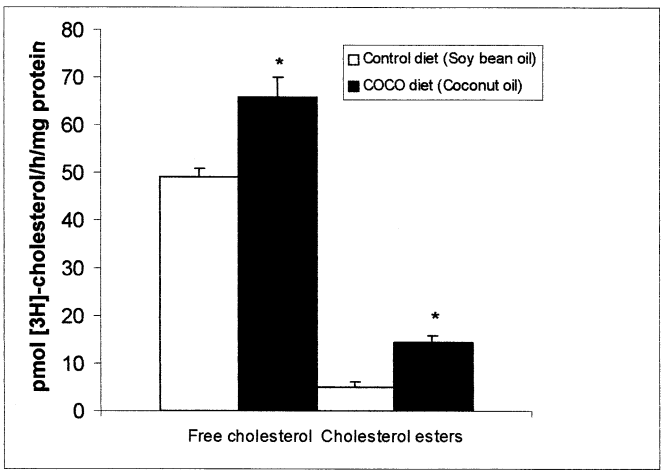

Fig. 2. Cholesterol incorporation in mouse peritoneal macrophages. The graph represents the means ± SEM from three experiments with three COCO- and control-fed mice each. Incorporation assay was made in two separate cell preparations by animal. *indicates significant differences ($p < 0.05$) between macrophages from mice fed the COCO diet and those fed the control diet.

3.3. Effect of the diet on phospholipid pattern in peritoneal macrophages

On the percentage basis the phospholipid composition was modified in macrophages of mice fed on the COCO diet compared with those fed on the control diet. In the COCO-fed group phosphatidylcholine and phosphatidylserine plus phosphatidylinositol increased and phosphatidylethanolamine decreased, while phosphatidylglycerol did not change in relation to the control-fed group, indicating that dietary saturated fat could modify the phospholipid compositions of resident peritoneal macrophages in mice (Fig. 1).

3.4. Incorporation of [^{3}H]-H_2O into macrophage

The incorporation of ^3H-H_2O into the macrophage lipids is shown in Table 4. In mice fed on the COCO diet, the ^{3}H incorporation into triglycerides and phospholipids increased while its incorporation into free cholesterol was lower than in the control-fed group. There was no difference in ^{3}H incorporation into esterified cholesterol between the dietary groups.

Table 4
Effect of the diets on the incorporation of ^{3}H from ^3H_2O into peritoneal macrophage lipids[1]

	(μmol ^{3}H/h/mg protein)		
	Control	COCO	
Free cholesterol	0.69 ± 0.05	0.50 ± 0.03	$p < 0.05$
Cholesterol esters	0.19 ± 0.07	0.26 ± 0.08	NS
Triglyceride	0.90 ± 0.05	1.12 ± 0.08	$p < 0.05$
Phospholipid	0.83 ± 0.05	1.03 ± 0.04	$p < 0.05$

[1] Values are means ± SEM for three experiments with three COCO- and control-fed mice each. Chemical determinations of each animal were made in two separate cell preparations. The data were analyzed by Student's *t*-test. The *P* values in the table indicate significant differences between feeding diet containing coconut oil (COCO) and soybean oil (control group). NS, not significant.

3.5. [^{3}H]-cholesterol incorporation and estimation of cholesterol esterification in vitro

The [^{3}H]-cholesterol incorporated by macrophages at 60 min of incubation was increased by 25% in the mice fed on the COCO diet when compared with that of mice fed on the control diet (Fig. 2). We could detect a significant increase in the radiolabeled cholesterol esters of macrophages from mice fed on the COCO diet (Fig. 2).

3.6. Effect of the diet on macrophage fatty acid compositions

The fatty acid composition of the lipid fraction of peritoneal macrophages is shown in Table 5. Feeding the COCO diet resulted in an increase in the proportion of myristic (14:0), palmitic (16:0) and palmitoleic acids [16:1(n-7)] and a decrease in linolenic [18:2 (n-6)] and arachidonic acids [(20:4 (n-6)), without changes in the proportion of stearic (18:0) and oleic acids [18:1(n-9)], compared with the control diet. The fatty acid composition of peritoneal macrophages shows that feeding the COCO diet resulted in a higher relative percentage of saturated fatty acids ($p < 0.001$) and in a lower unsaturation index ($p < 0.001$) compared with the control diet.

Table 5
Effect of the diet on peritoneal macrophage fatty acid compositions[1]

	Fatty acid (mol %)		
	Control	COCO	
14:0	3.34 ± 0.1	10.00 ± 0.5	$p < 0.001$
16:0	25.45 ± 0.4	34.94 ± 0.2	$p < 0.001$
16:1 (n-7)	1.29 ± 0.1	2.58 ± 0.1	$p < 0.001$
18:0	30.23 ± 0.8	31.08 ± 1.6	NS
18:1	13.19 ± 1.9	11.78 ± 0.9	NS
18:2 (n-6)	16.55 ± 1.0	4.67 ± 0.4	$p < 0.001$
18:3 (n-3)	0.30 ± 0.1	—	
20:4 (n-6)	9.65 ± 0.5	4.95 ± 0.7	$p < 0.01$
Σ SAT[2]	59.02 ± 0.8	76.02 ± 1.4	$p < 0.001$
Σ PUFA[3]	26.50 ± 0.3	9.62 ± 0.6	$p < 0.001$
UI[4]	0.87 ± 0.04	0.44 ± 0.01	$p < 0.001$

[1] Values are means ± SEM for three experiments with three COCO- and control-fed mice each.

[2] Sum of the saturated fatty acids.

[3] Sum of the polyunsaturated fatty acids.

[4] Unsaturation index: Sum of the porcentages of individual unsaturated fatty acid x number of double bonds/100. Means differences were determined by using Student's test. The *P* values in the table indicate significant differences between feeding diet containing coconut oil (COCO) and soybean oil (control group). NS, not significant.

4. Discussion

Lipid metabolism is very important in macrophage function, and could be a key target for direct and indirect modulating effects of exposure to the dietary fat. This study provides experimental evidence of the effect of saturated fat diet on lipid metabolism of resident peritoneal macrophages. The mouse fed on the COCO diet had significantly higher fasting plasma cholesterol and triglyceride concentrations compared with the controls. The increase of plasma cholesterol concentration is in agreement with previously published data in rats [26] and guinea pigs [27] fed on a diet enriched by saturated fatty acids. The hypercholesterolemia is associated with the increase of the level of plasma LDL + VLDL. In contrast, it has been shown that linoleic acid enriched diets have a hypocholesterolemic potential, which is due to the fact that cholesterol is taken up rapidly by the liver and other tissues [28]. The hypertriglyceridemia produced by the COCO diet could be caused by enhanced liver VLDL-triglyceride secretion into the circulation, since other authors have demonstrated an increase of hepatic VLDL production in guinea pigs fed on a diet containing 15% (w/w) palm kernel oil which contain more than 90% saturated fatty acids [29]. Simultaneously, mice fed on the COCO diet showed an increase in the lipoperoxidation process measured by plasma TBARS in relation to control animals. The oxidation of LDL has been associated to a decrease in the content of polyunsaturated fatty acids and an abundance of products of lipoperoxidation [30].

We observed important changes in lipid composition of macrophages obtained from mice fed on the COCO diet. Macrophages can acquire cholesterol both from endogenous synthesis and by uptake of cholesterol-containing extracellular material [4]. The increase in the incorporation of [^{3}H]cholesterol into macrophages of COCO-fed mice and also in the cholesterol esters fraction, in relation to macrophages of control mice, indicates that exogenous cholesterol is effectively uptaken and esterified by those cells. This is in agreement with previously published data showing that the accumulation of cholesterol in cells leads to an increase in the mRNA level of acyl coenzyme A:cholesterol acyltransferase (ACAT) [31]. Under normal conditions, peritoneal macrophages have a very low amount of esterified cholesterol but when these cells are activated its concentration increases [32]. The decrease in the ^{3}H incorporated into free cholesterol could suggest a feedback suppression of the cholesterol synthesis by the sterol end-products of the pathway. In addition, it has been shown that human monocyte-macrophages accumulate cholesterol ester in lipid droplets when they are incubated with microcystalline cholesterol or acetylated low density lipoprotein and that the presence of lipid droplets is consistent with the fact that macrophages contain triglycerides [3,33]. Likewise, we found an increase in the triglyceride concentration and in the ^3H_2O incorporation into triglycerides of macrophages from COCO-fed mice in relation to those from control-fed mice. This fact is probably a direct consequence of the metabolic changes produced by the COCO diet in the peritoneal macrophages. The higher content of total phospholipids found in macrophages of mice fed on the COCO diet is associated to the increase in PC and PS + PI and also agrees with the increase in ^3H_2O incorporation into the phospholipid fraction. PC is the major phospholipid in peritoneal macrophages [2] and it is known that free cholesterol increases the PC synthesis through activation of CTP:phosphocholine cytidylyltransferase (CTP-CT), an enzyme which regulates the PC synthesis [34]. Thus, the high concentration of free cholesterol observed in macrophages of COCO-fed mice may be responsible, at least in part, for the PC increase. The decrease in the PE content, as well as in other phospholipids, can be an adaptive cellular response to maintain the homeostasis of phospholipids [35].

The composition of fatty acids in inflammatory cells such as macrophages, in particular the content of polyunsaturated fatty acids, is very important for immuno-inflammatory functions, due to their role as substrate for the synthesis bio-active lipids, such as eicosanoids. The COCO diet altered the major fatty acyl constituents of macrophages. While the total saturated fatty acid contents increases, a significant decrease in the unsaturation index was observed, suggesting changes in membrane fluidity and, subsequently, in membrane function. The decrease in the relative percentage of unsaturated fatty acids is associated to the decrease in the linoleic acid and arachidonic acid contents, which is also in agreement with the low levels of polyunsaturated fatty acids of the COCO-diet. On the other hand, the low amount of arachidonic acid can be associated with the decrease in PE content of peritoneal macrophages

of mice fed on the COCO-diet. In vitro experiments in macrophages have demonstrated that the arachidonic acid is mainly incorporated into PE [36]. In addition, knowing that linoleic acid is the precursor of arachidonic acid, the diminution of linoleic acid content in peritoneal macrophages of mice fed on the COCO diet is probably a consequence of an activation of Δ^6 desaturase in order to maintain the arachidonic acid level. Although desaturase activity was not measured in this study, it is also conceivable that altered Δ^9 desaturase activity can be responsible for the significant increase in [16:1(n-7)] in macrophages of animals fed the high saturated fat diet.

In our experimental model we observed an increased TBARS concentration in macrophages and plasma from mice fed on the COCO-diet in relation to those fed on the control diet, indicating lipid peroxidation. It has been shown that fatty acid-rich diets with 20% by weight of hydrogenated coconut oil increase the production of reactive oxygen species in resident rodent macrophages [9,37]. Thus, it is possible that the oxidative stress process is operating in whole animals exposed to saturated fat in the diet. Other minor components of dietary oils such as tocopherols, which are low in coconut oil, could also have some effect on the susceptibility to oxidative stress. It has been shown that α-tocopheryl acetate supplementation in the diet decreases the iron-induced lipid peroxidation in tissues [38].

Our results are a demonstration of specific changes in the lipid metabolism and induction of lipid peroxidation of resident peritoneal macrophages when mice are fed on a saturated fat diet. Those facts could alter the immune-inflammatory function of peritoneal macrophages, either directly or through changes in membrane fluidity.

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