Effect of dietary fat saturation on lipid metabolism, arachidonic acid turnover and peritoneal macrophage oxidative stress in mice

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Abstract

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We investigated the effects of a saturated fat diet on lipid metabolism and arachidonic acid (AA) turnover in mouse resident peritoneal macrophages. The pro-oxidative effect of this diet was also studied. Female 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 (10 mice per group). The fat content of each diet was 15% (w/w). Mice were fed for 6 weeks and then sacrificed. The concentration of total lipids, triglycerides, (LDL + VLDL)-cholesterol, thiobarbituric acid-reactive substances (TBARS) and reduced glutathione were increased in the plasma of mice fed the COCO diet, without changes in phospholipid or total cholesterol concentrations compared to control. The concentrations of total cholesterol, free and esterified cholesterol, triglycerides, and TBARS were increased in the macrophages of COCO-fed mice, while the content of total phospholipids did not change. The phospholipid composition showed an increase of phosphatidylcholine and a decrease of phosphatidylethanolamine. The [³H]-AA distribution in the phospholipid classes showed an increase in phosphatidylcholine and phosphatidylethanolamine. Incorporation of [³H]-cholesterol into the macrophages of COCO-fed mice and into the cholesterol ester fraction was increased. The COCO diet did not affect [³H]-AA uptake but induced an increase in [³H]-AA release. The COCO diet also enhanced AA mobilization induced by lipopolysaccharide. These results indicate that the COCO diet, high in saturated fatty acids, alters the lipid metabolism and AA turnover of peritoneal macrophages in female mice and also produces a significant degree of oxidative stress.

Introduction

Macrophages are immunoinflammatory cells characterized by an active energetic, oxidative and lipid metabolism. Several func-tions of macrophages depend on their lipid composition, such as signal reception and transduction, phagocytosis, and synthesis of oxidized lipids and other inflammatory mediators (e.g., eicosanoids and platelet-acti-

vating factor) (1). The phospholipids of mouse peritoneal macrophages are highly enriched with arachidonic acid (AA) (2) and have a selective 85-kDa cytosolic phospholipase A_2 ($cPLA_2$), a phosphatide 2-acylhydrolase (EC 3.1.1.4) that hydrolyzes the sn-2 arachidonyl ester linked to phosphoglycerides, producing free AA and lysophospholipids (3). Free AA can also act as second messenger and as a substrate for inflammatory mediator synthesis (2). In macrophages, AA and reactive oxygen species (ROS) release is induced by diverse agonists including calcium ionophores, phorbol 12-myristate 13-acetate, okadaic acid, opsonized zymosan, and bacterial lipopolysaccharide (LPS) (4), and these releases are correlated with activation of $cPLA_2$ and NADPH oxidase (5). Macrophages play an important role in the processing of cholesterol derived from the metabolism of plasma lipoproteins as well as in the reutilization of cholesterol from damaged or senescent cells. There is a well-documented association between dietary saturated fat intake, elevated plasma LDL-cholesterol levels, and increased risk of cardiovascular disease (6,7).

It is known that changes in dietary fat can alter the tissue fatty acid composition and distribution but the extent and nature of these changes can vary markedly according to tissue site and cell type. The amount, not the position, of dietary 16:0 of coconut oil and palm oil determines the 16:0 content of piglet adipose tissue (8). Fatty acids play a role in modulating cell metabolism and signal transduction. Indeed, fatty acid-rich diets (with 20% by weight of hydrogenated coconut oil or unsaturated olive, safflower, or menhaden oil) 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 thioglycolate-elicited macrophages (9-11).

It is known that the oxidant-antioxidant balance is critical for immune cell function because it maintains the integrity and functionality of the cell membrane, cellular proteins and nucleic acids. Moreover, this balance is important in controlling signal transduction and gene expression. 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 ROS, 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 alter the mucosal cell membrane lipid composition in the rat large intestine and influences the susceptibility of mucosal cell lipid to peroxidation (13). Membrane lipids can be oxidized by an autocatalytic free radical reaction whereby polyunsaturated fatty acids and phospholipids undergo degradation by a chain reaction that results in the formation of lipoperoxides, various aldehydes (e.g., malondialdehyde, MDA), and short chain hydrocarbons (e.g., ethane, propane, pentane), among others (12). Oxidative stress induced by dietary saturated fatty acids can alter cell membranes, modifying the lipid composition and function of peritoneal macrophages (11,14). Glutathione (GSH), a cysteine-containing tripeptide, is the most abundant non-protein thiol in mammalian cells and plays an important role in the scavenging of ROS and free radicals and in the regulation of AA metabolism (15). Because redox status depends on the cellular balance between pro-oxidant and antioxidant factors, GSH could have an important effect on AA turnover in macrophages.

The major goal of this study was to investigate the effects of dietary saturated fat on the lipid composition, lipid peroxidation and AA turnover of female mouse resident peritoneal macrophages.

Material and Methods

Chemicals

Phenol red-free Dulbecco's modified Eagle's medium (DMEM) and fetal calf serum (FCS) were purchased from Gibco BRL (Life Technologies Inc., Rockville, MD, USA). [1,2-^{3}H(N)]-cholesterol (9.25 MBq/g) was purchased from Dupont (NEN™, New England Nuclear Life Science Products Inc., Boston, MA, USA). [5,6,8,9,11,12,14,15-^{3}H]-AA ([^{3}H]-delta4 AA, 185 Ci/mmol) was obtained from NEN™. Antibiotics, LPS from *Escherichia coli* serotype O55:B5 and lipid standards were acquired from Sigma (St. Louis, MO, USA). The plasma concentrations of total lipids, total cholesterol, HDL-cholesterol, phospholipids and triglyceride were determined by enzymatic methods using kits from Boehringer Mannheim Diagnostics (Indianapolis, IN, USA). All other chemicals were of reagent grade and were purchased from Merck Laboratory (Buenos Aires, Argentina).

Animals and diets

Adult female C57BL/6 mice bred in our animal facilities and maintained in a humidity- and temperature- (21-23ºC) controlled environment with a 12-h light-dark cycle were used. The mice came from dams 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. Mice were housed in individual stainless steel cages and given free access to food and water throughout the 6 weeks of the experimental period. Diets were isocaloric of identical compositions except for the fat type. The fat content was 15% (w/w) in both the control and COCO diets. The fatty acid composition of the diets was as follows: the control diet contained 12:0, 0.1%; 14:0, trace

($\leq$0.1%); 16:0, 9.1%; 16:1(n-7), 0.2%; 18:0, 5.9%; 18:1(n-9), 23.0%; 18:2(n-6), 53.2%; 18:3(n-3), 8.4%, and the COCO diet contained: 12:0, 41.6%; 14:0, 18.6%; 16:0, 10.9%; 18:0, 13.6%; 18:1(n-9), 2.7%; 18:2(n-6), 2.1%; 18:3(n-3), 0.2%. Coconut oil also contained approximately 8% caprylic (8:0) and 6% capric (10:0) acids (13), but fatty acids shorter than 12:0 were not resolved under our working conditions. Diets were prepared according to the AIN-93 for laboratory rodents (16). The oils were stored in opaque containers at -70ºC and the diets were prepared fresh daily by the addition of the appropriate amount of oil to a mix of the other dietary components. Body weight and food intake were recorded daily.

Plasma determinations

Mice were killed by decapitation between 8:00 and 9:00 am of the last experimental day. Blood was collected into EDTA-coated tubes (150 mg EDTA/l in 9 g NaCl/l), and plasma was prepared by centrifugation. Plasma lipids were determined by enzymatic methods using Boehringer kits and 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.

Isolation and culture of peritoneal macrophages

Resident peritoneal macrophages were prepared in an LPS-free system as described previously (17). Peritoneal cells were prepared by washing the peritoneal cavity with ice-cold Ca^{2+}-, Mg^{2+}- and phenol red-free Hank's balanced salt solution (HBSS) containing 1 mM EDTA and 1% BSA. After washing twice with cold HBSS, the peritoneal cells were suspended in DMEM and 2 x 10^6 cells were added to each well of 12-well culture plates (Corning Glass Works, Corning, NY, USA). After incubation for 2 h at

37ºC in a 95% air/5% CO_2 atmosphere, non-adherent cells were removed by vigorously washing three times with ice-cold HBSS. Differential counts of the adherent cells used for the experiments were determined micro-scopically after Giemsa and nonspecific es-terase staining. Resident preparations were 95% macrophages. Macrophages were fur-ther cultured for 24 h in phenol red-free DMEM supplemented with 10% FCS, 50 µg/ml gentamicin, 50 µg/ml penicillin, and 50 µg/ml fungizone. The amount of macro-phage protein of each well was measured by the method of Wang and Smith (18).

To obtain the macrophage suspensions and determine cell viability, adherent cells were harvested by incubation at 4ºC with 1 ml of cold 5 mM EDTA/5% FCS in HBSS. The cell suspension was aspirated and cen-trifuged at 200 g for 10 min at 4ºC and the cells were then washed with cold HBSS. The pellet was resuspended in 1 ml HBSS and 10 µl of the cell suspension 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 light microscopy. Cell viability evaluated by Trypan blue ex-clusion was never below 95%. When neces-sary, 250 µl of the washed cell suspension (4 x 10^6 cells/ml of HBSS) was treated with 250 µl of lysis solution (20 mM Tris-succi-nate, pH 7.8, containing a protease inhibitor cocktail and 0.1% Triton X-100).

Lipid determinations

A macrophage suspension containing 4 x 10^6 cells was centrifuged and lipids were extracted with chloroform:methanol (2:1) from the cell pellet according to the method of Folch et al. (19). An aliquot of the lipid extracts was used to determine total choles-terol, and another one to separate the differ-ent lipid fractions (phospholipids, triglycer-ide, free cholesterol and esterified choles-terol) by thin-layer chromatography (TLC) using plates coated with silica gel G (Merck),

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 Rouser et al. (20) for phospholipids, of Sardesai and Man-ning (21) for triglyceride, and of Zak et al. (22) for free and esterified cholesterol. On average, 90% of the cholesterol mass was recovered by TLC. Phospholipids were sepa-rated into component species by TLC using silica gel G plates and chloroform:methanol:water (65:25:4, v/v) as the solvent system. The individual phospholipids were identi-fied, recovered and quantified for phospho-rus content as indicated above. The results are reported as percentage of total phospho-lipid phosphorus content. The position of neutral lipids and individual phospholipids was determined using the respective standards.

Incorporation of [³H]-cholesterol by peritoneal macrophages

After 24 h of culture the peritoneal mac-rophages (2 x 10^6 cells) were incubated with 0.125 µCi of [³H]-cholesterol for 60 min at 37ºC in a 95% air/5% CO_2 atmosphere. Li-pid extraction and separation of cholesterol and cholesterol ester bands were carried out by TLC as indicated above. Bands were scraped off and their radioactivity was meas-ured with a Beckman LS 100 C liquid scintil-lation counter (ESANCO SRL, Buenos Aires, Argentina). The results are reported as pmol [³H] incorporated h⁻¹ mg protein⁻¹.

Arachidonic acid uptake and release

Macrophage monolayers (2 x 10^6 cells) were washed three times with HBSS and incubated in 1 ml DMEM containing 1 µCi [³H]-delta4 AA for 30, 60, 120, 240 and 360 min at 37ºC. The cell cultures were then washed with HBSS containing 0.5% BSA to remove non-incorporated [³H]-AA. At the

end of each incubation the macrophage monolayers were treated with lysis solution as described earlier (see Isolation and culture of peritoneal macrophages). Finally, the radioactivity present in the solubilized cells was measured by scintillation counting.

In the AA release experiments, cell monolayers were labeled with [3H]-delta4 AA for 6 h at 37ºC. The macrophages used in these studies incorporated $41 \pm 5\%$ of [3H]-AA added to the culture medium, and no significant variations in cells from COCO-fed mice were observed compared to those from mice receiving the control diet. The cell monolayers were then washed three times with HBSS containing 0.5% BSA to remove non-incorporated [3H]-AA and stimulated in 1 ml HBSS containing 10 mM glucose, 1 mM $CaCl_2$, 1 mM $MgCl_2$, antibiotics, 0.5% BSA (to prevent AA re-esterification) and LPS (1 µg/ml). After 3 h of incubation, the medium was removed and the amount of radioactivity in the cells and medium was measured as indicated above. The released radioactivity was determined and is reported as percent cell-incorporated [3H]-AA, which was determined in solubilized cells.

Arachidonic acid distribution in phospholipid classes

After 6 h of incubation in medium containing 0.1 µCi [3H]-delta4 AA, total lipids from macrophage monolayers were extracted and total and individual phospholipids were separated by TLC (see Lipid determinations). Their radioactivity was measured as described above. The [3H]-AA of individual phospholipids is reported as percent [3H]-AA incorporated into total phospholipids.

TBARS and GSH determinations

Thiobarbituric acid-reactive substances (TBARS) were measured as described by Jentzsch et al. (23). Briefly, serum (200 µl)

or standard (MDA prepared by hydrolysis of tetramethoxypropane) was mixed with 25 µl butylated hydroxytoluene in ethanol (0.3 µmol/l) and 200 µl orthophosphoric acid (0.2 µmol/l). Thiobarbituric acid reagent (25 µl at 0.11 mol/l, dissolved in NaOH) was added and the reaction mixture was incubated at 90ºC for 45 min. TBARS were extracted with 500 µl n-butanol. Absorption was read at 535 and 572 nm to correct for baseline absorption in a Beckman microplate reader (Bio-Rad, Alfatron, Buenos Aires, Argentina). MDA equivalents were calculated using the difference in absorption at the two wavelengths and quantified with the aid of a calibration curve. Total GSH (GSH + GSSG) was extracted from macrophages with 10% perchloric acid. The GSH content in the cells was measured using an enzymatic method (24), which is based on the catalytic action of GSH in the reduction of 5,5'-dithiobis (2-nitrobenzoic acid) by means of the GSH reductase system. The GSH extracted from cells was mostly GSH and the content of the oxidized form (GSSG) was less than 10% of total GSH under the present experimental conditions.

Statistical analysis

Data are reported as mean $\pm$ SD and were analyzed statistically by the Student t-test for noncorrelated data. Statistical significance was accepted at $P < 0.05$.

Results

Growth, food intake and plasma concentrations of lipids, TBARS and GSH

The initial body weight of the mice in both dietary groups was the same (8 ± 0.6 g). At the time of sacrifice, there was no significant effect of the saturated fatty acid dietary manipulation on the body weight gain of the mice fed the COCO diet compared to that of mice fed the control diet (12.70 ± 0.82 *vs*

14.01 ± 0.61 g) after 6 weeks of dietary treatment. As the mice grew the daily food consumption increased similarly in both dietary groups (data not shown). Plasma total lipids, (LDL + VLDL)-cholesterol fraction

Table 1. Effect of the diet on plasma lipids, reduced glutathione (GSH) and thiobarbituric acid reactive substances (TBARS).

	Control diet	COCO diet
Plasma lipids (g/l)		
Total lipids	5.38±0.22	7.48±0.19*
Triglyceride	1.49±0.08	2.15±0.17*
Total cholesterol	0.88±0.06	1.02±0.07
HDL-cholesterol	0.57±0.05	0.54±0.04
(LDL + VLDL)-cholesterol	0.31±0.03	0.48±0.04*
Phospholipids	0.79±0.04	0.80±0.06
TBARS (nmol MDA/ml)	6.52±0.78	10.55±1.03*
GSH (µEq/ml)	0.88±0.02	1.34±0.03*

Data are reported as means ± SEM for 10 mice/group. NS = not significant.
*P < 0.01 compared to the control diet (Student *t*-test).

Table 2. Effect of the diet on peritoneal macrophage lipid composition and thiobarbituric acid reactive substances (TBARS).

	Control diet	COCO diet
Lipids (µg/mg protein)		
TC	86.20±3.24	117.53±5.20*
FC	78.74±1.80	105.98±4.79*
EC	7.46±0.46	11.55±0.50*
Triglyceride	73.08±1.87	90.22±2.05*
Phospholipids	219.46±8.77	225.10±10.25
FC/EC	10.51±0.42	9.13±0.81
TC/phospholipids	0.40±0.08	0.52±0.09
Protein (mg/10[7] cells)	1.09±0.14	1.12±0.17
TBARS (nmol MDA/mg protein)	6.72±0.57	10.88±0.42*

Data are reported as means ± SEM of four experiments with three COCO- and control-fed mice each. Chemical determinations were performed on two separate cell preparations from each animal. EC = esterified cholesterol; FC = free cholesterol; TC = total cholesterol.
*P < 0.01 compared to the control diet (Student *t*-test).

and triglyceride concentrations were significantly higher in COCO-fed mice than in controls. There were no differences in plasma phospholipid concentrations between the dietary groups. The plasma TBARS and GSH levels were significantly higher in the COCO-fed group compared to control (P < 0.001; Table 1).

Effect of the diet on the macrophage lipid composition and TBARS content

As shown in Table 2, the concentration of total cholesterol (which was related to the increased free and esterified cholesterol fractions) and triglyceride increased in the peritoneal macrophages of COCO-fed mice compared to control. Consequently, the free cholesterol/esterified cholesterol ratio did not change. In addition to the changes in lipid composition of macrophages of mice fed the COCO diet, cell TBARS levels also increased compared to those of mice fed the control diet. Cell protein concentration was not affected by the diet.

Effect of the diet on the phospholipid pattern in peritoneal macrophages

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

[3H]-cholesterol incorporation and estimation of cholesterol esterification *in vitro*

The [3H]-cholesterol incorporated by

macrophages at 60 min of incubation was increased by 28% in the mice fed the COCO diet when compared with that of mice fed the control diet. We detected a significant increase in the radiolabeled cholesterol esters of macrophages from mice fed the COCO diet (Figure 2).

Arachidonic acid uptake and release and distribution into phospholipid classes

The incorporation of labeled AA by peritoneal macrophages of both dietary groups showed a time-dependent increase up to 360 min. The COCO diet did not affect the uptake of [^{3}H]-AA (Figure 3). Peritoneal macrophages from COCO-fed mice caused a significant increase in [^{3}H]-AA release in the absence of LPS in the incubation medium with respect to control cells. The addition of LPS to the medium caused a marked increase in [^{3}H]-AA release from macrophages from COCO-fed mice compared to the respective control cells (Figure 4). Macrophages of COCO-fed and control mice did not show significant variations in AA incorporation into total phospholipids (data not shown). The distribution of [^{3}H]-AA into the major classes of phospholipids demonstrated that macrophages of COCO-fed mice incorporate more label in PC and PE than those of control macrophages (Figure 5).

Discussion

The present results provide experimental evidence for the effects of coconut oil in the diet on AA turnover, lipid peroxidation and lipid contents in resident peritoneal macrophages from female mice. Mice fed the COCO diet had significantly higher fasting plasma total lipids compared to control, which is associated with the increase of triglyceride levels. High plasma triglyceride concentrations have been demonstrated in subjects consuming a saturated fat diet (25) and have also been associated with the VLDL
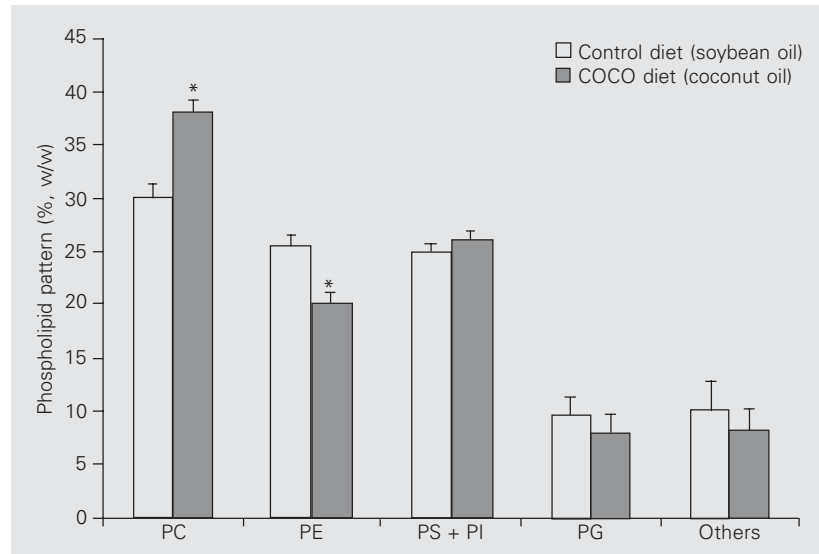

Figure 1. Phospholipid pattern of mouse peritoneal macrophages. PC = phosphatidylcholine; PE = phosphatidylethanolamine; PG = phosphatidylglycerol; PS + PI = phosphatidylserine plus phosphatidylinositol. Data are reported as means ± SEM of three experiments with three COCO- and control-fed mice each. Chemical determinations were performed on two separate cell preparations from each animal. *P < 0.05 compared to the control diet (Student *t*-test).
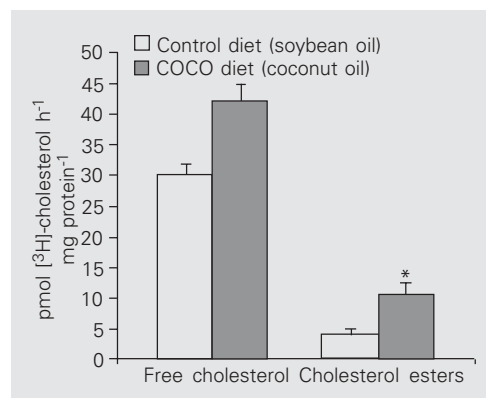

Figure 2. Cholesterol incorporation in mouse peritoneal macrophages. Data are reported as means ± SEM of three experiments with three COCO- and control-fed mice each. The incorporation assay was performed on two separate cell preparations per animal. *P < 0.05 compared to control (Student *t*-test).
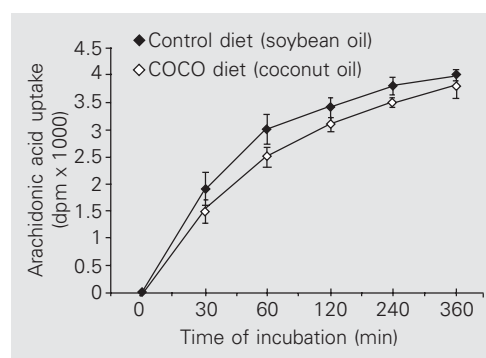

Figure 3. Kinetics of arachidonic acid uptake by peritoneal macrophages from mice fed the COCO diet and those fed the control diet. Data are reported as means ± SEM of three experiments in duplicate.

levels in hamsters fed a 14:0-enriched diet (26). The hypertriglyceridemia produced by the COCO diet could be caused by enhanced liver VLDL-triglyceride secretion into the circulation, since other investigators have demonstrated an increase of hepatic VLDL production in guinea pigs fed a diet containing 15% (w/w) palm kernel oil, which con-

tains more than 90% saturated fatty acids (27). In contrast, it has been shown that linoleic acid-enriched diets have a hypocholesterolemic potential due to the fact that cholesterol is taken up rapidly by the liver and other tissues (28).

The COCO diet induced important changes in the lipid composition of peritoneal macrophages. Macrophages can acquire cholesterol both by endogenous synthesis and by uptake of cholesterol-containing extracellular material (7). The increase in the incorporation of [^{3}H]-cholesterol into macrophages of COCO-fed mice and also in the cholesterol ester fraction compared to control indicates that exogenous cholesterol is effectively taken up and esterified by these cells. This is in agreement with 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) (29). Also, a significant fraction of arachidonate released from phospholipids, in particular from PC, could serve as substrate for ACAT in rat peritoneal macrophages (30). Accordingly, there was an increase of [^{3}H]-AA mobilization in macrophages of COCO-fed mice. Under normal conditions, peritoneal macrophages contain a very small amount of esterified cholesterol but this amount increases when macrophages are activated (31). The decrease in [^{3}H] incorporated into free cholesterol suggests a feedback suppression of 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 microcrystalline cholesterol or acetylated LDL and that the presence of lipid droplets is consistent with the fact that macrophages contain triglycerides (6,32).

Similarly, we found an increase in triglyceride concentration in macrophages from COCO-fed mice compared to those from control mice. This is probably a direct conse-

Figure 4. Arachidonic acid mobilization from peritoneal macrophages. Cells were incubated in DMEM containing 1 μCi [^{3}H]-arachidonic acid for 6 h and then exposed to LPS for 3 h. Arachidonic acid mobilization was measured as described in Material and Methods. Data are reported as means ± SEM of three experiments with three COCO- and control-fed mice each, using two separate cell preparations per animal. *P < 0.001 compared to medium without LPS; +P < 0.001 compared to the control diet (two-way ANOVA).
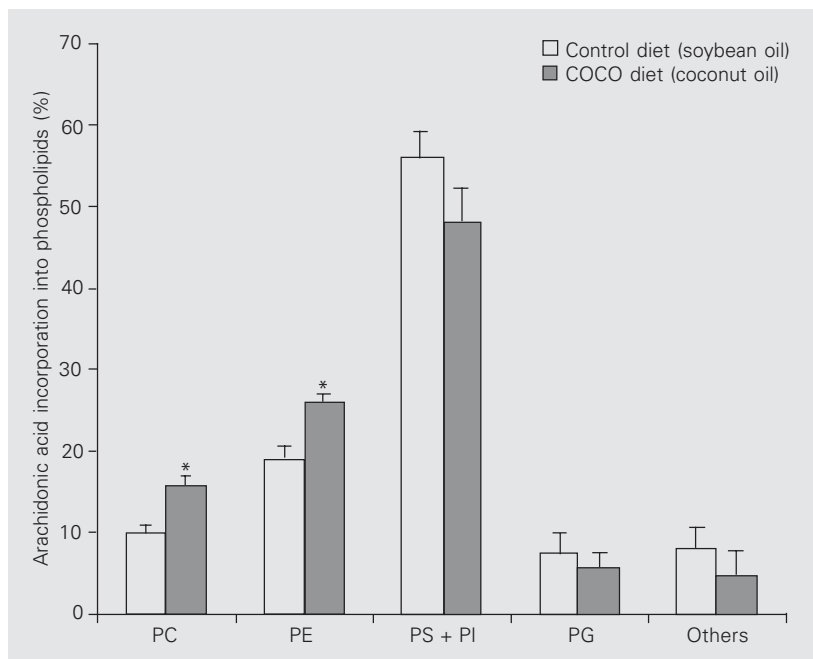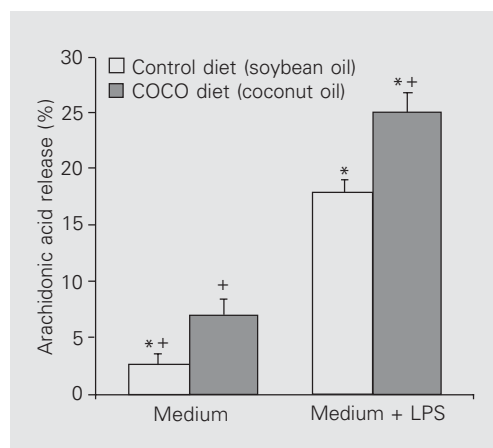

Figure 5. Arachidonic acid incorporation into the major phospholipid classes. PC = phosphatidylcholine; PE = phosphatidylethanolamine; PG = phosphatidylglycerol; PS + PI = phosphatidylserine plus phosphatidylinositol. Data are reported as means ± SEM of three experiments with three COCO- and control-fed mice each. Chemical determinations were performed on two separate cell preparations per animal. *P < 0.001 compared to the control diet (Student t-test).

quence of the metabolic changes induced by the COCO diet in the peritoneal macrophages. PC is the major phospholipid in peritoneal macrophages (2) and it is known that free cholesterol increases PC synthesis by activation of cytosine triphosphate:phosphocholine cytidylyltransferase (CTP-CT), an enzyme which regulates PC synthesis (33). 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 PE content and also the distribution of [^{3}H]-AA into PC and PE can be an adaptive cellular response in order to maintain phospholipid homeostasis. It has been demonstrated that in P388D1 macrophage-like cells the phospholipid pool constitutes the primary metabolic fate of the delta4-AA via a remodeling pathway (34).

The increased TBARS concentration in macrophages and plasma from mice fed the COCO diet in relation to those fed the control diet may be associated with lipid peroxidation. TBARS have been included in the products of peroxidation, which are considered to depend on the basic oxidizable lipid/antioxidant relationship (35). Therefore, we cannot exclude the possibility that the increase in TBARS may be associated with the increase in lipid content induced by the COCO diet. Additionally, plasma GSH levels were increased in these animals. It has been shown that fatty acid-rich diets containing 20% by weight of hydrogenated coconut oil increase the production of ROS in resident rodent macrophages (36). In addition, it is known that mammalian cells respond to oxidative stress by several mechanisms, including GSH production (37). GSH can react with electrophilic or oxidizing spe-

cies, protects protein thiol groups from oxidation, serves as substrate for many enzymes including GSH peroxidase, GSH reductase and GSH-S-transferase, and may participate in the detoxification of ROS. Thus, in our experimental model, the increase in plasma GSH may reflect an adaptive change in response to oxidative stress induced by a saturated fat diet. It is possible that the oxidative stress process is operating in animals exposed to saturated fat in the diet as a whole.

The increase in AA release observed in macrophages of COCO-fed mice suggests that activated macrophages would lead to an increased availability of free AA, which can be used to synthesize eicosanoids (1). In contrast, it has been shown that fish oil diets induce a decrease in [^{3}H]-AA mobilization in rat resident peritoneal macrophages treated with proinflammatory agents (38). In addition, the oxidative stress induced by exposure of vascular smooth muscle cells to H_2O_2 increases AA release (39). Gijón and Leslie (5) reported that AA release and eicosanoid production in response to agonists are dramatically reduced in resident peritoneal macrophages isolated from $cPLA_2$ knockout mice. Furthermore, the role of $cPLA_2$ in the mobilization of fatty acid damage by oxidative stress in cell membranes has been considered to be a cellular response to oxidative stress stimuli (40).

The present findings show that coconut oil in the diet modifies AA turnover and lipid metabolism and also provide evidence of the pro-oxidative effect of this saturated fat diet on the peritoneal macrophages of female mice. These metabolic consequences of the diet may alter the immunoinflammatory function of peritoneal macrophages, either directly or through changes in membrane fluidity.

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