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Enhanced Aortic Macrophage Lipid Accumulation and Inflammatory Response in LDL Receptor Null Mice Fed an Atherogenic Diet

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Abstract

The effect of an atherogenic diet on inflammatory response and elicited peritoneal macrophage (M ϕ) cholesterol accumulation in relation to aortic lesion formation was assessed in LDL receptor null (LDLr $^{-/-}$) mice. Mice were fed an atherogenic or control diet for 32 weeks. The atherogenic relative to control diet resulted in significantly higher plasma monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor alpha (TNF α) and interleukin-6 (IL-6) concentrations, more aortic wall M ϕ deposition, higher serum non HDL-cholesterol concentrations and total cholesterol to HDL-cholesterol ratios, and greater accumulation of both aortic free and esterified cholesterol. Elicited peritoneal M ϕ selectively accumulated longer chain unsaturated fatty acids in their membrane, independent of the dietary fatty acid profile. Elicited peritoneal M ϕ isolated from mice fed the atherogenic relative to control diet had significantly less arachidonic acid levels, accumulated significantly higher esterified cholesterol, had significantly higher mRNA levels and secretion of MCP-1, and mRNA and protein levels of ATP-binding cassette A1. Diet treatment

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had no significant effect in elicited peritoneal M ϕ on TNF α and IL-6 mRNA levels and secretion. These data suggest that the atherogenic relative to control diet resulted in higher plasma inflammatory factor concentrations, less favorable lipoprotein profile, higher elicited peritoneal M ϕ cholesterol accumulation and inflammatory factor secretion, and more aortic wall M ϕ deposition, which in turn were associated with greater aortic cholesterol accumulation.

Keywords

Atherogenic diet; Atherosclerosis; Cholesterol accumulation; Inflammatory response; Macrophage; LDLr $^{-/-}$ mouse

Introduction

Historically, lipoprotein profiles and lipid accumulation in the blood vessel wall have been the major focus of research related to atherogenesis. More recently, a preponderance of evidence from clinical and experimental studies has suggested that inflammation is an important factor [1]. Macrophages (M ϕ) play a critical role in both intracellular cholesterol accumulation and deposition in the arterial wall. Aortic M ϕ s express scavenger receptors that take up modified lipoproteins through membrane-bound M ϕ scavenger receptor 1 (MSR1) and CD36. Increased expression of MSR1 and CD36 facilitates the uptake of modified lipoproteins [2,3]. Two M ϕ membrane proteins involved in cholesterol efflux are ATP-binding cassette A1 (ABCA1) and scavenger receptor B class 1 (SR-B1) [4]. When the M ϕ cholesterol influx is greater than the efflux, cholesterol homeostasis in M ϕ is disturbed, and cholesterol accumulation is favored. The resulting M ϕ -derived foam cells produce reactive oxygen species, which in turn modify lipoproteins, and secrete pro-inflammatory factors, which amplify the local inflammatory reaction [1].

Exposure of endothelial cells to inflammatory factors results in the expression of monocyte chemoattractant protein-1 (MCP-1) and its receptor, CC chemokine receptor 2 (CCR2), which direct the migration of monocytes into the intimal wall. Subsequent exposure of the monocytes to M ϕ colony-stimulating factor causes differentiation to M ϕ . Overexpression of MCP-1 is positively associated with monocyte accumulation in fatty streaks [5]. Major pro-inflammatory factors include interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF α). They are synthesized by monocytes/M ϕ and several other types of cells, such as T cells, endothelial cells and adipocytes, and in turn stimulate the synthesis of other inflammatory factors [6,7]. Dietary fatty acids and cholesterol have been shown to alter the concentrations of these biomarkers of inflammation and the atherogenic process [8–10].

We used the LDL receptor null (LDLr $^{-/-}$) mouse fed diets high in saturated fat and cholesterol (atherogenic diet) or low in those components (control diet) as an animal model to assess the relationship between aortic lesion formation and the inflammatory response. Our hypothesis was that the atherogenic diet would accelerate aortic lipid accumulation due to induction of an atherogenic lipoprotein profile, elevate plasma inflammatory biomarker concentrations, increase cholesterol accumulation in and inflammatory factor secretion from elicited peritoneal M ϕ , and change expression and protein levels of genes associated with these processes.

Experimental Procedures

Animals and Diets

Twenty-two 8-week-old, male LDLr $^{-/-}$ mice (Jackson Laboratory, Bar Harbor, Maine) initially weighing 20.2 ± 2.8 g were placed in individual cages with stainless-steel wire

bottoms in a windowless room maintained at 22–24°C, 45% relative humidity and a daily 10/14 light/dark cycle with the light period from 0600 to 1600 hours. After 1 week of acclimation, mice were weighed and randomly assigned to one of two groups. The mice were fed either a control diet low in butterfat (4% fat, w/w, 9% energy) and cholesterol (0.02%, w/w) or an atherogenic diet high in butterfat (20% fat, w/w, 38% energy) and cholesterol (0.2%, w/w) ad libitum. The atherogenic diet was modified from Harlan Teklad atherogenic diet TD.88137. The composition of the diets is provided in Supplementary Table 1, and the fatty acid profile of the diets is provided in Supplementary Table 2. Body weight and food intake were recorded weekly over a 32-week period. In order to ensure adequate quantities of M ϕ for the proposed work, 5 days prior to killing, mice were given an intraperitoneal injection of 1.0 ml Brewer thioglycollate broth (4.05 g/100 ml) to elicit peritoneal M ϕ accumulation [11]. Immediately after a 16–18 h fast, the mice were anesthetized with CO₂ and killed by exsanguination. Blood was collected by retro-orbital bleeding to harvest serum at weeks 0, 12 and 32 and by cardiac puncture to harvest plasma at week 32. Heart, aorta and elicited peritoneal M ϕ were collected and treated as described in the next section. Serum and plasma were obtained by blood centrifugation at 1,100×g at 4°C for 25 min. The animal protocol was approved by the Animal Care and Use Committee of the Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, and was in accordance with guidelines provided by the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Data from the mice fed the atherogenic diet were used for comparison purposes to address an unrelated experimental question [12].

Atherosclerosis Lesion Quantitation

The mouse hearts were perfused in situ for 1 min with diethylpyrocarbonate treated-phosphate-buffered saline (PBS) containing 1.5 μ mol/l aprotinin and 0.1 mmol/l phenylmethylsulfonyl fluoride through a cannula inserted into the left ventricle. Eight randomly chosen aortas were dissected from the aortic root to the iliac bifurcation using a stereoscopic zoom microscope. As a measure of atherosclerotic lesion, total cholesterol (TC) and free cholesterol (FC) were quantified (8 aortas/group) as previously described [13,14]. Esterified cholesterol (EC) was calculated as the difference between the two measures. The residual delipidated aortic tissue was digested in 1N NaOH, and total protein was determined using a bicinchoninic acid (BCA) kit (Pierce Ins., Rockford, IL).

Immunohistochemistry

In each group of mice, three randomly chosen aortic arches, from the left aortic valve to the right subclavian artery branch, were embedded in OCT compound (Tissue-Tek 4583; Sakura Finetek), snap-frozen in liquid nitrogen and stored at –80°C until sectioning. The aortic arches were sectioned at a 5- μ m thickness and stained in the pathology facility at New England Medical Center. Rat anti-mouse monocyte/M ϕ antibody (MCA519G; Serotec, Raleigh, NC) was used to identify monocyte/M ϕ deposition.

Serum Lipid Profile

Serum TC, HDL-cholesterol (HDL-C) and triglyceride concentrations were measured using an Olympus AU400 analyzer with enzymatic reagents (Olympus America, Melville, NY). Non-HDL-cholesterol (non HDL-C) was calculated as the difference between TC and HDL-C [15].

Plasma Concentrations of Inflammatory Factors

Plasma TNF α , IL-6 and MCP-1 concentrations were measured using Quantikine[®] ELISA kits (R&D Systems, Minneapolis, MN).

Elicited Peritoneal M ϕ Culture and Stimulation

Immediately after harvesting, elicited peritoneal M ϕ were cultured in RPMI1640 medium (ATCC, Manassas, VA) containing 2% heat-inactivated fetal bovine serum (Invitrogen, Carlsbad, CA) for 2 h (1×10^6 cells per ml). One million cells were stimulated with 1 μ g/ml lipopolysaccharide (LPS) for 15 h, and inflammatory factors released into the culture medium were measured. The remaining cells were washed with PBS three times and used for the measurement of cholesterol content, fatty acid profile, mRNA and protein levels of genes involved in inflammation and cholesterol accumulation.

Cholesterol content and fatty acid profile: elicited peritoneal M ϕ TC, FC and protein concentrations were determined as described above for the aorta. M ϕ fatty acid profiles were determined by gas chromatography as previously described [16].

Secretion of inflammatory factors: TNF α , IL-6 and MCP-1 secretion from LPS-stimulated elicited peritoneal M ϕ were measured using DuoSet[®] ELISA kits (R&D Systems, Minneapolis, MN). Elicited peritoneal M ϕ attached to the culture plates were digested with 0.5N NaOH, and total protein was determined using BCA kits (Pierce Ins., Rockford, IL).

Western blot and real-time PCR: protein was extracted from elicited peritoneal M ϕ using RIPA kit (Santa Cruz, Santa Cruz, CA). Western blotting was performed as previously described [15] with the following primary antibodies: SR-B1 (Novus Biologicals, Littleton, CO), ABCA1 (Novus Biologicals, Littleton, CO), CD36 (Cascade Bioscience, Winchester, MA) and β -actin (Sigma, St. Louis, MO). Signals were visualized by chemiluminescence (Amersham Biosciences, Piscataway, NJ) and quantified using a GS-800 calibrated densitometer (Bio-Rad, Hercules, CA).

RNA was extracted from M ϕ using RNeasy mini kit (Qiagen, Valencia, CA). cDNA was synthesized from RNA using SuperScript[™] II reverse transcriptase according to the manufacturer's instruction (Invitrogen, Carlsbad, CA). Primers for peroxisomal proliferator activated receptor (PPAR) β , PPAR γ , MSR1, CD36, SR-B1, ABCA1, MCP-1, TNF α and β -actin (Table 1) were designed using Primer Express version 2.0 (Applied Biosystems, Foster City, CA). β -actin was used as an endogenous control. Primer amplification efficiency and specificity were verified for each set of primers. cDNA levels of the genes of interest were measured using power SYBR green master mix on real-time PCR 7300 (Applied Biosystems, Foster City, CA). cDNA levels of IL-6 and PPAR α were measured using Taqman[®] gene expression assays and Taqman[®] PreAmp master mix kit (Applied Biosystems, Foster City, CA). The reaction condition was 95°C for 10 min, 40 cycles of 95°C for 15 s, 60°C for 1 min and one cycle of dissociation stage. mRNA fold change was calculated using the $2^{-[\Delta\Delta C(T)]}$ method [17].

Statistical Methods

Prior to statistical testing, data were checked for normality, and appropriate transformations were made when necessary (PROC UNIVARIATE; SAS version 9.1, SAS Institute Inc, Cary, NC). Unpaired Student's *t* test (PROC TTEST) was performed to compare group means. Differences were considered significant at $P < 0.05$. Untransformed data are presented in text, figures and tables as mean $\pm$ standard deviation (SD).

Results

Animal Body Weight and Food Intake

With one exception at the 10-week feeding point, there was no significant difference in the body weight between mice fed the two experimental diets during the 32-week feeding period

(data not shown). Although the weight of food eaten was higher in the mice fed the control diet, on the basis of energy intake, the two groups were similar.

Aortic Lesion Composition

Shown in Fig. 1a is a representative aorta from each group of mice. Chemical composition of eight aortas per group indicated that mean TC was 5.5-fold ($P < 0.001$) higher in mice fed the atherogenic compared to control diet (Fig. 1b). This was contributed by higher amounts of both FC (4.9-fold, $P < 0.001$) and EC (6.4-fold, $P < 0.001$). There was stronger staining for M ϕ in the cross sections of aortic arch isolated from the atherogenic compared to control diet-fed mice (Fig. 1c).

Serum Lipid Profile

Although similar at baseline, serum non-HDL-C ($P < 0.001$) concentrations and ratios of TC to HDL-C ($P < 0.001$) were significantly higher in the atherogenic relative to control diet-fed mice at the 12-week feeding point, and these differences were maintained throughout the study period (Fig. 2). By the end of the 32-week feeding period, serum HDL-C concentrations declined similarly between the two groups of mice, 35 and 28% in mice fed the control and atherogenic diets, respectively. During that same time period, triglyceride concentrations increased by 94 and 139% in the mice fed the control and atherogenic diets, respectively.

Plasma Concentrations of Inflammatory Factors

At the end of the 32-week feeding period, the mice fed the atherogenic relative to control diet had significantly higher plasma concentrations of all three of the inflammatory factors assessed: MCP-1 (144%, $P < 0.01$), TNF α (325%, $P < 0.01$) and IL-6 (188%, $P < 0.001$) (Fig. 3).

Elicited Peritoneal M ϕ Fatty Acid Profiles

The total SFA, monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) profile of the elicited peritoneal M ϕ was similar between the two diet groups. With the exception of small differences, the percentage of omega (ω)-6 PUFA ($P < 0.01$) was significantly lower, whereas that of ω -3 PUFA ($P < 0.001$) was significantly higher in the elicited peritoneal M ϕ isolated from the atherogenic relative to control diet-fed mice (Table 2). Although alpha-linolenic acid (ALA, C18:3 ω -3) levels were higher than eicosapentaenoic acid (EPA, C20:5 ω -3) and docosahexaenoic acid (DHA, C22:6 ω -3) in both diets, DHA plus EPA levels were 18-fold higher than ALA in elicited peritoneal M ϕ isolated from the two groups of mice. Although linoleic acid (LNA, C18:2 ω -6) levels were more than 60-fold higher than arachidonic acid (ARA, C20:4 ω -6) in both diets, ARA levels were 1.8-fold and 1.4-fold higher than LNA in elicited peritoneal M ϕ isolated from mice fed the control and atherogenic diet, respectively.

Elicited Peritoneal M ϕ Cholesterol Content

To examine the potential relationship between aortic and M ϕ cholesterol accumulation, we measured elicited peritoneal M ϕ cholesterol content. Elicited peritoneal M ϕ TC was 34% ($P < 0.01$) higher in the atherogenic relative to control diet-fed mice (Fig. 4). This was accounted for primarily by the EC fraction, which was 104% ($P < 0.01$) higher in the atherogenic diet-fed mice.

mRNA and Protein Levels of Genes Involved in Cholesterol Accumulation in Elicited Peritoneal M ϕ

To investigate the mechanism(s) underlying the higher EC content in the elicited peritoneal M ϕ harvested from the mice fed the atherogenic diet, we measured the protein and mRNA levels of genes involved in cholesterol accumulation. The atherogenic diet resulted in significantly higher ABCA1 mRNA ($P < 0.01$) and protein ($P < 0.01$) levels, albeit a modest difference, whereas SR-B1 and CD36 mRNA and protein levels were similar between two diet groups (Fig. 5a, c). There was no significant effect of diet treatment on mRNA levels of MSR1, PPAR α , PPAR β/δ and PPAR γ .

mRNA Levels and Secretion of Inflammatory Factors in/From Elicited Peritoneal M ϕ

To explore whether plasma concentrations of inflammatory factors were determined by M ϕ secretion, we measured MCP-1, TNF α and IL-6 mRNA levels and secretion in/from elicited peritoneal M ϕ . MCP-1 secretion was 51% higher ($P < 0.01$) in mice fed the atherogenic relative to control diet (Fig. 5b). This was accompanied by 1.5-fold higher MCP-1 mRNA levels ($P < 0.05$) (Fig. 5c). There was no significant effect of diet treatment on TNF α and IL-6 mRNA levels in and secretion from elicited peritoneal M ϕ —suggesting these cells were not a major determinant of plasma TNF α and IL-6 concentrations.

Discussion

In humans, consumption of high SFA and cholesterol diets is positively associated with cardiovascular disease (CVD) risk [18,19]. The inflammatory process is a major contributor to the development of atherosclerotic plaque [1,20]. IL-6, MCP-1 and TNF α are important biomarkers of inflammation [6,21,22]. Epidemiological studies have shown a positive association between the Western-type diet and plasma MCP-1, IL-6 and TNF α concentrations [23–25]. The present study is the first to simultaneously assess the effect of an atherogenic diet in the LDLr $^{-/-}$ mouse on atherosclerotic lesion formation, aortic M ϕ accumulation, plasma inflammatory factor concentrations, cholesterol accumulation and inflammatory factor secretion in/from elicited peritoneal M ϕ isolated from these mice, and M ϕ gene expression and protein levels associated with these processes. We demonstrated that diet-induced atherosclerotic lesion formation in LDLr $^{-/-}$ mice was associated with higher inflammatory response. Changes in peritoneal M ϕ isolated from these mice may, in part, have exacerbated the aortic lesion formation.

LNA and ALA were the most abundant ω -6 fatty acids, and accounted for more than 84 and 79% of total ω -6 and ω -3 fatty acids in two diets, respectively. ARA only accounted for 0.2 and 1.4% of total ω -6 fatty acids in the control and atherogenic diet, respectively. However, ARA accounted for about 41.5 and 38.7% of total ω -6 fatty acids in elicited peritoneal M ϕ isolated from the control and atherogenic diet-fed mice, respectively. The percentage of LNA was about one third less than that of ARA in elicited peritoneal M ϕ isolated from two groups of mice. ALA represented about 3% of total ω -3 fatty acids in elicited peritoneal M ϕ . EPA and DHA were the most abundant ω -3 fatty acids and accounted for more than 56% of total ω -3 fatty acids in elicited peritoneal M ϕ . These data suggest that peritoneal M ϕ selectively accumulated longer chain unsaturated fatty acids in their membrane, independent of the fatty acid profile of the diet. Similar data have been reported by others [26]. Whereas the total SFA, MUFA and PUFA profile of the elicited peritoneal M ϕ was similar between the two groups of mice, the ω -6 PUFAs were significantly higher, and ω -3 PUFAs were significantly lower in elicited peritoneal M ϕ isolated from the control relative to atherogenic diet-fed mice. ARA accounted for about 38.4 and 28.3% of total PUFA in elicited peritoneal M ϕ isolated from the control and atherogenic diet-fed mice, respectively. EPA plus DHA accounted for about 5.3 and 15.3% of total PUFA in elicited peritoneal M ϕ isolated from the

control and atherogenic diet-fed mice, respectively. ARA was significantly lower and EPA plus DHA significantly higher in elicited peritoneal M ϕ isolated from the atherogenic relative to control diet-fed mice. Further studies are required to detect whether these differences are a result of uptake of cholesterol-rich lipoproteins or specific fatty acid synthesis caused by diets or inflammation.

Overexpression of MCP-1 has been positively related to monocyte accumulation in the arterial vessel wall, a major event leading to the development of atherosclerosis [5,27]. Studies in humans have shown that elevated serum MCP-1 concentrations are an inflammatory biomarker positively associated with CVD [27–29]. In MCP-1 and LDL receptor double null mice, lower monocyte deposition and vessel wall lipid accumulation have been observed compared to LDLr^{-/-} mice [30,31]. LDLr^{-/-} mice fed a Western diet had elevated plasma MCP-1 concentrations, which were associated with more aortic lesion area compared to the mice fed chow diet [32]. In this study, in elicited peritoneal M ϕ , both MCP-1 mRNA levels and secretion were significantly higher in LDLr^{-/-} mice fed the atherogenic diet compared to those fed the control diet. This is consistent with previous reports that a similar atherogenic diet resulted in higher MCP-1 mRNA levels in and protein secretion from adipocytes in mice [32–34]. Although the atherogenic diet resulted in elevated plasma IL-6 and TNF α concentrations, there was no significant difference in mRNA and secretion of TNF α and IL-6 from elicited peritoneal M ϕ isolated from two groups of mice. TNF α and IL-6 can be produced by liver, endothelial, adipocytes or T cells [35–37]. Preliminary work suggested that liver TNF α and IL-6 production was 1.5-fold ($P < 0.05$) and 2.0-fold higher ($P < 0.05$), respectively, in mice fed the atherogenic relative to control diet despite higher plasma concentrations of these cytokines (unpublished data). These data suggest that elevated plasma IL-6 and TNF α concentrations were mediated, at least in part, by liver cells.

Apolipoprotein E-deficient mice fed a high SFA and cholesterol diet had significantly higher MCP-1 mRNA levels in both liver and aorta compared to mice fed only a high SFA diet [38]. The atherogenic diet might increase the expression of MCP-1 through upregulated Toll-like receptor 4 (TLR4). Stimulation of TLR4 by LPS can induce activation of nuclear factor-kappa B (NF- κ B) and expression of cytokines [39]. TLR4 is expressed in murine and human lipid-rich atherosclerotic lesions, and colocalized with M ϕ both in murine atherosclerotic lesion and human shoulder coronary artery plaque [40]. In vitro studies have shown that oxidized LDL (ox-LDL) can upregulate the expression of TLR4 in human monocyte-derived M ϕ [40]. We cannot rule out the possibility that high levels of ox-LDL in the atherogenic diet-fed mice stimulated the expression of TLR4, which increased the binding of LPS, and further upregulated the expression of MCP-1. Due to the limited number of elicited peritoneal M ϕ obtained from each mouse, we could not test the above hypotheses in this study.

In this study, ARA levels were significantly higher than EPA plus DHA in the elicited peritoneal M ϕ isolated from two groups of mice. In addition, the percentage of ARA was significantly higher, whereas that of EPA plus DHA were significantly lower, in the elicited peritoneal M ϕ isolated from the control relative to atherogenic diet-fed mice. Our previous study [41] has shown that ARA significantly lowered MCP-1 mRNA levels and secretion from M ϕ differentiated from THP-1 monocytes. ARA and EPA had a more pronounced effect than DHA. ARA can be metabolized to 15-deoxy-delta^{12,14}-PGJ₂ (15dPGJ₂), a natural ligand for PPAR γ [42]. 15dPGJ₂ has been consistently shown to have anti-inflammatory properties by serving as a potent agonist to activate PPAR γ [43,44]. Some studies have shown that activated PPAR γ could be a promoter-specific repressor of NF- κ B target genes that regulate inflammation in M ϕ [45]. There was not a significant difference in the PPAR γ expression in the elicited peritoneal M ϕ isolated from two groups of mice. ARA

might lower MCP-1 expression through activating PPAR γ instead of changing its expression.

The atherogenic diet resulted in more M ϕ deposition in the aortic wall. In situ, these cells play an important role not only in the inflammatory response, but also in uptake of modified LDL, and accumulation and deposition of cholesterol [46,47]. Based on the results from previous studies [11,48,49], the expression and production of inflammatory factors is comparable between resident and elicited peritoneal M ϕ . In our study, elicited peritoneal M ϕ were used to ensure an adequate number of cells were harvested. Elicited peritoneal M ϕ TC and EC content change was similar to that seen in the aortas of the respective mice. To determine whether the difference in M ϕ cholesterol content resulting from the two different diets was due to an imbalance of influx or efflux, we assessed mRNA and protein levels of two genes involved in cholesterol efflux, SR-B1 and ABCA1, and two genes involved in cholesterol influx, MSR1 (only mRNA levels) and CD36. There was no significant difference in protein levels of CD36 and SR-B1 in elicited peritoneal macrophages isolated from the two groups of mice. An antibody to detect protein levels of MSR1 was not available. The atherogenic diet resulted in higher mRNA and protein levels of ABCA1 in this study, but not the other factors assessed. These data are consistent with other studies showing that M ϕ ABCA1 expression is upregulated in atherosclerotic lesions, as a compensatory mechanism in response to cholesterol enrichment [50,51]. ABCA1 is a direct transcriptional target of liver-X-receptors (LXR) [52–54]. LXR is activated by cholesterol metabolites. We speculate that higher circulating lipo-protein concentrations, especially apolipoprotein B-containing lipoproteins, would favor increased M ϕ cholesterol content, which in turn would alter cellular cholesterol homeostasis and trigger an upregulation of ABCA1 expression. The atherogenic diet may have activated LXR through increasing M ϕ production of LXR ligands, such as 22(R)-hydroxycholesterol and 27-hydroxycholesterol. Ligand activated-LXR can upregulate ABCA1 gene expression and enhance ABCA1-mediated cholesterol efflux from elicited peritoneal M ϕ isolated from the atherogenic diet-fed mice. In addition to the cholesterol enrichment-induced regulation, it has been reported that SFA increase and PUFA decrease ABCA1 expression at both transcription and post-translation levels [55,56]. However, there was no significant difference in SFA and PUFA composition in elicited peritoneal M ϕ isolated from two groups of mice. The higher levels of TC and EC in the peritoneal M ϕ may have resulted from higher circulation oxLDL concentrations, upregulation of genes associated with cholesterol influx or/and downregulation of genes associated with cholesterol efflux, which were not determined due to limited numbers of M ϕ harvested from each mouse.

In summary, these data suggest that LDLr $^{-/-}$ mice fed a diet high in SFA and cholesterol, relative to the control diet, had higher plasma inflammatory factor concentrations, less favorable lipoprotein profiles, higher elicited peritoneal M ϕ cholesterol accumulation and inflammatory factor secretion, and more M ϕ deposition in the aortic wall, which in turn were associated with greater aortic cholesterol accumulation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

Mϕ	Macrophage
LDLr^{-/-}	LDL receptor null
MCP-1	Monocyte chemoattractant protein-1
TNFα	Tumor necrosis factor alpha
IL-6	Interleukin-6
MSR1	Membrane bound M ϕ scavenger receptor 1
CD36	Cluster of differentiation 36
ABCA1	ATP-binding cassette A1
SR-B1	Scavenger receptor B class 1
CCR2	CC chemokine receptor 2
PBS	Phosphate-buffered saline
TC	Total cholesterol
FC	Free cholesterol
EC	Esterified cholesterol
BCA	Bicinchoninic acid
HDL-C	High density lipoprotein-cholesterol
non HDL-C	Non-high density lipoprotein-cholesterol
LPS	Lipopolysaccharide
SD	Standard deviation
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid
ALA	Alpha-linolenic acid
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
LNA	Linoleic acid
ARA	Arachidonic acid
CVD	Cardiovascular disease
TLR4	Toll-like receptor 4
NF-κB	Nuclear factor-kappa B
ox-LDL	Oxidized LDL
LXR	Liver-X-receptors

References

1. Willerson JT, Ridker PM. Inflammation as a cardiovascular risk factor. *Circulation*. 2004; 109:II2–II10. [PubMed: 15173056]

2. Lam MC, Tan KC, Lam KS. Glycoxidized low-density lipoprotein regulates the expression of scavenger receptors in THP-1 macrophages. *Atherosclerosis*. 2004; 177:313–320. [PubMed: 15530905]
3. Moore KJ, Freeman MW. Scavenger receptors in atherosclerosis: beyond lipid uptake. *Arterioscler Thromb Vasc Biol*. 2006; 26:1702–1711. [PubMed: 16728653]
4. Baranova I, Vishnyakova T, Bocharov A, Chen Z, Remaley AT, Stonik J, Eggerman TL, Patterson AP. Lipopolysaccharide down regulates both scavenger receptor B1 and ATP binding cassette transporter A1 in RAW cells. *Infect Immun*. 2002; 70:2995–3003. [PubMed: 12010990]
5. Vita JA, Keaney JF Jr, Larson MG, Keyes MJ, Massaro JM, Lipinska I, Lehman BT, Fan S, Osypiuk E, Wilson PW, Vasan RS, Mitchell GF, Benjamin EJ. Brachial artery vasodilator function and systemic inflammation in the Framingham Offspring Study. *Circulation*. 2004; 110:3604–3609. [PubMed: 15569842]
6. Fiotti N, Giansante C, Ponte E, Delbello C, Calabrese S, Zacchi T, Dobrina A, Guarnieri G. Atherosclerosis and inflammation. Patterns of cytokine regulation in patients with peripheral arterial disease. *Atherosclerosis*. 1999; 145:51–60. [PubMed: 10428295]
7. Haddy N, Sass C, Drosch S, Zaiou M, Siest G, Ponthieux A, Lambert D, Visvikis S. IL-6, TNF- α and atherosclerosis risk indicators in a healthy family population: the STANISLAS cohort. *Atherosclerosis*. 2003; 170:277–283. [PubMed: 14612208]
8. Seierstad SL, Seljeflot I, Johansen O, Hansen R, Haugen M, Rosenlund G, Froyland L, Arnesen H. Dietary intake of differently fed salmon; the influence on markers of human atherosclerosis. *Eur J Clin Invest*. 2005; 35:52–59. [PubMed: 15638820]
9. Meydani M. Omega-3 fatty acids alter soluble markers of endothelial function in coronary heart disease patients. *Nutr Rev*. 2000; 58:56–59. [PubMed: 10748610]
10. Basu A, Devaraj S, Jialal I. Dietary factors that promote or retard inflammation. *Arterioscler Thromb Vasc Biol*. 2006; 26:995–1001. [PubMed: 16484595]
11. Wu D, Marko M, Claycombe K, Paulson KE, Meydani SN. Ceramide-induced and age-associated increase in macrophage COX-2 expression is mediated through up-regulation of NF-kappa B activity. *J Biol Chem*. 2003; 278:10983–10992. [PubMed: 12529332]
12. Wang S, Wu D, Matthan NR, Lamon-Fava S, Lecker JL, Lichtenstein AH. Reduction in dietary omega-6 polyunsaturated fatty acids: eicosapentaenoic acid plus docosahexaenoic acid ratio minimizes atherosclerotic lesion formation and inflammatory response in the LDL receptor null mouse. *Atherosclerosis*. 2009; 204:147–155. [PubMed: 18842266]
13. Rudel LL, Kelley K, Sawyer JK, Shah R, Wilson MD. Dietary monounsaturated fatty acids promote aortic atherosclerosis in LDL receptor-null, human ApoB100-overexpressing transgenic mice. *Arterioscler Thromb Vasc Biol*. 1998; 18:1818–1827. [PubMed: 9812923]
14. Matthan NR, Giovanni A, Schaefer EJ, Brown BG, Lichtenstein AH. Impact of simvastatin, niacin, and/or antioxidants on cholesterol metabolism in CAD patients with low HDL. *J Lipid Res*. 2003; 44:800–806. [PubMed: 12562840]
15. Dorfman SE, Wang S, Vega-Lopez S, Jauhiainen M, Lichtenstein AH. Dietary fatty acids and cholesterol differentially modulate HDL cholesterol metabolism in Golden-Syrian hamsters. *J Nutr*. 2005; 135:492–498. [PubMed: 15735083]
16. Lichtenstein AH, Matthan NR, Jalbert SM, Resteghini NA, Schaefer EJ, Ausman LM. Novel soybean oils with different fatty acid profiles alter cardiovascular disease risk factors in moderately hyperlipidemic subjects. *Am J Clin Nutr*. 2006; 84:497–504. [PubMed: 16960162]
17. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{(-Delta Delta C(T))} method. *Methods*. 2001; 25:402–408. [PubMed: 11846609]
18. Millen BE, Quatromoni PA, Nam BH, O'Horo CE, Polak JF, D'Agostino RB. Dietary patterns and the odds of carotid atherosclerosis in women: the Framingham Nutrition Studies. *Prev Med*. 2002; 35:540–547. [PubMed: 12460521]
19. Klor HU, Hauenschild A, Holbach I, Schnell-Kretschmer H, Stroh S. Nutrition and cardiovascular disease. *Eur J Med Res*. 1997; 2:243–257. [PubMed: 9182652]
20. Paoletti R, Gotto AM Jr, Hajjar DP. Inflammation in atherosclerosis and implications for therapy. *Circulation*. 2004; 109:III20–III26. [PubMed: 15198962]

21. Brueckmann M, Bertsch T, Lang S, Sueselbeck T, Wolpert C, Kaden JJ, Jaramillo C, Huhle G, Borggreffe M, Haase KK. Time course of systemic markers of inflammation in patients presenting with acute coronary syndromes. *Clin Chem Lab Med*. 2004; 42:1132–1139. [PubMed: 15552272]
22. Hoogeveen RC, Morrison A, Boerwinkle E, Miles JS, Rhodes CE, Sharrett AR, Ballantyne CM. Plasma MCP-1 level and risk for peripheral arterial disease and incident coronary heart disease: atherosclerosis risk in communities study. *Atherosclerosis*. 2005; 183:301–307. [PubMed: 16285993]
23. Pawlak K, Pawlak D, Mysliwiec M. Inflammation but not oxidative stress is associated with beta-chemokine levels and prevalence of cardiovascular disease in uraemic patients. *Cytokine*. 2006; 35:258–262. [PubMed: 17070067]
24. Baer DJ, Judd JT, Clevidence BA, Tracy RP. Dietary fatty acids affect plasma markers of inflammation in healthy men fed controlled diets: a randomized crossover study. *Am J Clin Nutr*. 2004; 79:969–973. [PubMed: 15159225]
25. Lopez-Garcia E, Schulze MB, Fung TT, Meigs JB, Rifai N, Manson JE, Hu FB. Major dietary patterns are related to plasma concentrations of markers of inflammation and endothelial dysfunction. *Am J Clin Nutr*. 2004; 80:1029–1035. [PubMed: 15447916]
26. Miles EA, Wallace FA, Calder PC. Dietary fish oil reduces intercellular adhesion molecule 1 and scavenger receptor expression on murine macrophages. *Atherosclerosis*. 2000; 152:43–50. [PubMed: 10996338]
27. Ballantyne CM, Nambi V. Markers of inflammation and their clinical significance. *Atheroscler Suppl*. 2005; 6:21–29. [PubMed: 15823493]
28. Martinovic I, Abegunewardene N, Seul M, Vosseler M, Horstick G, Buerke M, Darius H, Lindemann S. Elevated monocyte chemoattractant protein-1 serum levels in patients at risk for coronary artery disease. *Circ J*. 2005; 69:1484–1489. [PubMed: 16308496]
29. Charo IF, Taubman MB. Chemokines in the pathogenesis of vascular disease. *Circ Res*. 2004; 95:858–866. [PubMed: 15514167]
30. Ishibashi M, Egashira K, Zhao Q, Hiasa K, Ohtani K, Ihara Y, Charo IF, Kura S, Tsuzuki T, Takeshita A, Sunagawa K. Bone marrow-derived monocyte chemoattractant protein-1 receptor CCR2 is critical in angiotensin II-induced acceleration of atherosclerosis and aneurysm formation in hypercholesterolemic mice. *Arterioscler Thromb Vasc Biol*. 2004; 24:e174–e178. [PubMed: 15331433]
31. Gu L, Okada Y, Clinton SK, Gerard C, Sukhova GK, Libby P, Rollins BJ. Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein receptor-deficient mice. *Mol Cell*. 1998; 2:275–281. [PubMed: 9734366]
32. Tian J, Pei H, Sanders JM, Angle JF, Sarembock IJ, Matsumoto AH, Helm GA, Shi W. Hyperlipidemia is a major determinant of neointimal formation in LDL receptor-deficient mice. *Biochem Biophys Res Commun*. 2006; 345:1004–1009. [PubMed: 16712797]
33. Chen A, Mumick S, Zhang C, Lamb J, Dai H, Weingarth D, Mudgett J, Chen H, MacNeil DJ, Reitman ML, Qian S. Diet induction of monocyte chemoattractant protein-1 and its impact on obesity. *Obes Res*. 2005; 13:1311–1320. [PubMed: 16129712]
34. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, Kitazawa S, Miyachi H, Maeda S, Egashira K, Kasuga M. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J Clin Invest*. 2006; 116:1494–1505. [PubMed: 16691291]
35. Charo IF, Ransohoff RM. The many roles of chemokines and chemokine receptors in inflammation. *N Engl J Med*. 2006; 354:610–621. [PubMed: 16467548]
36. Glass CK, Witztum JL. Atherosclerosis the road ahead. *Cell*. 2001; 104:503–516. [PubMed: 11239408]
37. Vignali DA, Collison LW, Workman CJ. How regulatory T cells work. *Nat Rev Immunol*. 2008; 8:523–532. [PubMed: 18566595]
38. Tous M, Ferre N, Rull A, Marsillach J, Coll B, Alonso-Villaverde C, Camps J, Joven J. Dietary cholesterol and differential monocyte chemoattractant protein-1 gene expression in aorta and liver of apo E-deficient mice. *Biochem Biophys Res Commun*. 2006; 340:1078–1084. [PubMed: 16403442]

39. Vink A, Schoneveld AH, van der Meer JJ, van Middelaar BJ, Sluijter JP, Smeets MB, Quax PH, Lim SK, Borst C, Pasterkamp G, de Kleijn DP. In vivo evidence for a role of toll-like receptor 4 in the development of intimal lesions. *Circulation*. 2002; 106:1985–1990. [PubMed: 12370224]
40. Xu XH, Shah PK, Faure E, Equils O, Thomas L, Fishbein MC, Luthringer D, Xu XP, Rajavashisth TB, Yano J, Kaul S, Ardit M. Toll-like receptor-4 is expressed by macrophages in murine and human lipid-rich atherosclerotic plaques and upregulated by oxidized LDL. *Circulation*. 2001; 104:3103–3108. [PubMed: 11748108]
41. Wang S, Wu D, Lamon-Fava S, Matthan NR, Honda KL, Lichtenstein AH. In vitro fatty acid enrichment of macrophages alters inflammatory response and net cholesterol accumulation. *Br J Nutr*. 2009; 102:497–501. [PubMed: 19660150]
42. Scher JU, Pillinger MH. 15d-PGJ2: the anti-inflammatory prostaglandin? *Clin Immunol*. 2005; 114:100–109. [PubMed: 15639643]
43. Jiang C, Ting AT, Seed B. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. *Nature*. 1998; 391:82–86. [PubMed: 9422509]
44. Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature*. 1998; 391:79–82. [PubMed: 9422508]
45. Pascual G, Fong AL, Ogawa S, Gamliel A, Li AC, Perissi V, Rose DW, Willson TM, Rosenfeld MG, Glass CK. A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-gamma. *Nature*. 2005; 437:759–763. [PubMed: 16127449]
46. Kunjathoor VV, Febbraio M, Podrez EA, Moore KJ, Andersson L, Koehn S, Rhee JS, Silverstein R, Hoff HF, Freeman MW. Scavenger receptors class A-I/II and CD36 are the principal receptors responsible for the uptake of modified low density lipoprotein leading to lipid loading in macrophages. *J Biol Chem*. 2002; 277:49982–49988. [PubMed: 12376530]
47. Ludwig B, Laman JD. The in and out of monocytes in atherosclerotic plaques: Balancing inflammation through migration. *Proc Natl Acad Sci USA*. 2004; 101:11529–11530. [PubMed: 15292506]
48. Hayek MG, Mura C, Wu D, Beharka AA, Han SN, Paulson KE, Hwang D, Meydani SN. Enhanced expression of inducible cyclooxygenase with age in murine macrophages. *J Immunol*. 1997; 159:2445–2451. [PubMed: 9278337]
49. Wu D, Mura C, Beharka AA, Han SN, Paulson KE, Hwang D, Meydani SN. Age-associated increase in PGE2 synthesis and COX activity in murine macrophages is reversed by vitamin E. *Am J Physiol*. 1998; 275:C661–668. [PubMed: 9730949]
50. Langmann T, Klucken J, Reil M, Liebisch G, Luciani MF, Chimini G, Kaminski WE, Schmitz G. Molecular cloning of the human ATP-binding cassette transporter 1 (hABC1): evidence for sterol-dependent regulation in macrophages. *Biochem Biophys Res Commun*. 1999; 257:29–33. [PubMed: 10092505]
51. Lawn RM, Wade DP, Garvin MR, Wang X, Schwartz K, Porter JG, Seilhamer JJ, Vaughan AM, Oram JF. The Tangier disease gene product ABC1 controls the cellular apolipoprotein-mediated lipid removal pathway. *J Clin Invest*. 1999; 104:R25–31. [PubMed: 10525055]
52. Laffitte BA, Repa JJ, Joseph SB, Wilpitz DC, Kast HR, Mangelsdorf DJ, Tontonoz P. LXRs control lipid-inducible expression of the apolipoprotein E gene in macrophages and adipocytes. *Proc Natl Acad Sci USA*. 2001; 98:507–512. [PubMed: 11149950]
53. Fu X, Menke JG, Chen Y, Zhou G, MacNaul KL, Wright SD, Sparrow CP, Lund EG. 27-hydroxycholesterol is an endogenous ligand for liver X receptor in cholesterol-loaded cells. *J Biol Chem*. 2001; 276:38378–38387. [PubMed: 11504730]
54. Beyea MM, Heslop CL, Sawyez CG, Edwards JY, Markle JG, Hegele RA, Huff MW. Selective up-regulation of LXR-regulated genes ABCA1, ABCG1, and APOE in macrophages through increased endogenous synthesis of 24(S), 25-epoxycholesterol. *J Biol Chem*. 2007; 282:5207–5216. [PubMed: 17186944]
55. Uehara Y, Miura S, von Eckardstein A, Abe S, Fujii A, Matsuo Y, Rust S, Lorkowski S, Assmann G, Yamada T, Saku K. Unsaturated fatty acids suppress the expression of the ATP-binding cassette transporter G1 (ABCG1) and ABCA1 genes via an LXR/RXR responsive element. *Atherosclerosis*. 2007; 191:11–21. [PubMed: 16730733]

56. Murthy S, Born E, Mathur SN, Field FJ. Liver-X-receptor-mediated increase in ATP-binding cassette transporter A1 expression is attenuated by fatty acids in CaCo-2 cells: effect on cholesterol efflux to high-density lipoprotein. *Biochem J.* 2004; 377:545–552. [PubMed: 14604434]

Fig. 1. Representative aortas from each diet group; white areas denote atherosclerotic lesion (a); aortic cholesterol content in whole aortas (b), and M ϕ immunostaining by an antibody to mouse M ϕ surface marker in the cross-sections of aortic arches (c) in LDLr^{-/-} mice. Magnified ($\times 200$) images of aortic arch cross sections. The intensity of the *brown color* corresponds to the presence of M ϕ . $n = 11$ in each group. Values are mean $\pm$ SD. Unpaired Student's t test (PROC TTEST) was performed to compare two group values. The difference is expressed as *** $P < 0.001$

Fig. 2.

Serum lipid profiles in LDLr^{-/-} mice. Serum was obtained at 0-, 12- and 32-week post-diet interventions. $n = 11$ in each group. Values are mean $\pm$ SD of untransformed data. Prior to the statistical analysis the following transformations were made: TG at 0 week (*inverse*), HDL at 12 week (*square*), non-HDL-C at 12 week (*inverse*), TC/HDL-C at 32 week (*square*). Unpaired Student's *t* test (PROC TTEST) was performed to compare two group values. The difference is expressed as ** $P < 0.01$, *** $P < 0.001$

Fig. 3.

Plasma concentrations of inflammatory factors in LDLr^{-/-} mice. $n = 11$ in each group. Values are mean $\pm$ SD of untransformed data. Prior to the statistical analysis, the following transformations were made: MCP-1 (inverse) and IL-6 (inverse). Unpaired Student's t test (PROC TTEST) was performed to compare two group values. The difference is expressed as ** $P < 0.01$, *** $P < 0.001$

Fig. 4.

Elicited peritoneal M ϕ cholesterol content in LDLr^{-/-} mice. $n = 11$ in each group. Values are mean $\pm$ SD of untransformed data. Prior to the statistical analysis, FC (inverse) was transformed. Unpaired Student's t test (PROC TTEST) was performed to compare two group values. The difference is expressed as ** $P < 0.01$, *** $P < 0.001$

Fig. 5. Protein levels of ABCA1, SR-B1 and CD36 (a), secretion of MCP-1, TNF α and IL-6 (b), and mRNA levels of genes (c) in/from elicited peritoneal M ϕ isolated from LDLR $^{-/-}$ mice at the end of the 32-week feeding period. $n = 11$ in each group. Values are mean $\pm$ SD of untransformed data. Prior to the statistical analysis, mRNA levels of TNF α [$1/\log(\text{TNF}\alpha)$] were transformed. Unpaired Student's t test (PROC TTEST) was performed to compare two group values. The difference is expressed as * $P < 0.05$, ** $P < 0.01$

Table 1

Mouse oligonucleotide sequences of primers

Gene name	Accession no.	Forward primer	Reverse primer
ABCA1	NM_013454	CCTGCTAAAATACCGCAAGG	GTAACCCGTTCCCAACTGGTTT
SR-B1	NM_016741	TGGAACGGA CT CAGCAAGATC	AATTCCAGCGAGGA TTCGG
CD36	NM_007643	ATTAATGGCACAGACGCAGC	CCGAACACAGCGTAGATAGACC
MSR1	AF203781	TCTACAGCAAAGCAACAGGAGG	TCCACGTGCGCTTGTTCTT
TNF α	NM_013693	TGTAGCCACGTCGTAGCAAA	GCTGGCACC ACTAGTTGGTTGT
MCP-1	NM_011333	TCTCTCTTCCTCCACCACCATG	GCGTTAACTGCATCTGGCTGA
PPAR β/δ	NM_011145	AGTGCGATCGGATCTGCAAGA	TCCAAAGCGGATAGCGTTGTG
PPAR γ	NM_011146	TCTTAACTGCCGGATCCACAAA	CCAAACCTGATGGCATTGTGA
β -Actin	NM_007393	CTTTTCCAGCCTTCCTTCTTGG	CAGCACTGTGTTGGCATAGAGG

Table 2

Peritoneal Mφ membrane fatty acid profile (mol %)

Fatty acid	Diet	
	Control ^a	Atherogenic ^b
SFA	37.58 ± 6.43	34.74 ± 6.21
C12:0	0.31 ± 0.15	0.38 ± 0.34
C14:0	1.10 ± 0.10	1.86 ± 0.21 ***
C16:0	24.33 ± 4.56	21.51 ± 3.38
C18:0	11.48 ± 2.11	10.60 ± 3.09
C20:0	0.15 ± 0.10	0.14 ± 0.07
C22:0	0.10 ± 0.03	0.10 ± 0.07
C24:0	0.12 ± 0.02	0.14 ± 0.04
MUFA	38.38 ± 3.51	42.54 ± 6.01
C16:1 ω -9	1.05 ± 0.15	1.30 ± 0.06 **
C16:1 ω -7	4.17 ± 0.68	4.04 ± 1.27
C18:1 ω -9	24.43 ± 2.82	28.69 ± 5.47
C18:1 ω -7	6.44 ± 0.59	5.57 ± 1.20
C20:1 ω -9	0.88 ± 0.07	1.13 ± 0.19 **
C22:1 ω -9	0.27 ± 0.08	0.31 ± 0.12
C24:1 ω -9	0.62 ± 0.18	0.66 ± 0.28
PUFA	24.04 ± 4.73	22.72 ± 2.49
ω -6	22.20 ± 4.16	16.61 ± 1.91 **
C18:2	5.15 ± 0.88	4.68 ± 0.86
C18:3	0.03 ± 0.03	0.02 ± 0.01
C20:2	1.38 ± 0.17	1.03 ± 0.14 ***
C20:3	1.23 ± 0.30	1.31 ± 0.30
C20:4	9.22 ± 2.17	6.43 ± 1.17 **
C22:2	0.96 ± 0.26	1.34 ± 1.42
C22:4	4.18 ± 1.16	1.67 ± 0.43 ***
C22:5	0.05 ± 0.05	0.15 ± 0.19
ω -3	1.84 ± 0.62	6.11 ± 0.67 ***
C18:3	0.07 ± 0.02	0.19 ± 0.04 ***
C20:5	0.21 ± 0.19	1.03 ± 0.73 ***
C22:5	0.50 ± 0.12	2.45 ± 0.38 ***
C22:6	1.07 ± 0.40	2.44 ± 0.69 ***

$n = 11$ in each group. Values are mean $\pm$ SD of untransformed data. Prior to the statistical analysis, the following transformations were made: C16:0 (square), C20:1 (inverse), C20:2 (inverse), C20:5 ω -3 (square root), C22:2 (1/square root), C22:5 ω -6 (square root), PUFA (inverse), ω -6 PUFA (inverse). Unpaired Student's t test (PROC TTEST) was performed to compare two group values. Within a row the difference is expressed as

**
 $P < 0.01$,

 $P < 0.001$

^a Control: low saturated fatty acid and low cholesterol diet

^b Atherogenic: high saturated fatty acid and high cholesterol diet