Omega-6 polyunsaturated fatty acids prevent atherosclerosis development in LDLr-KO mice, in spite of displaying a pro-inflammatory profile similar to trans fatty acids


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A B S T R A C T

The development of atherosclerosis and the inflammatory response were investigated in LDLr-KO mice on three high-fat diets (40% energy as fat) for 16 weeks: trans (TRANS), saturated (SAFA) or ω-6 polyunsaturated (PUFA) fats. The following parameters were measured: plasma lipids, aortic root total cholesterol (TC), lesion area (Oil Red-O), ABCA1 content and macrophage infiltration (immunohistochemistry), collagen content (Picrosirius-red) and co-localization of ABCA1 and macrophage (confocal microscopy) besides the plasma inflammatory markers (IL-6, TNF-α) and the macrophage inflammatory response to lipopolysaccharide from Escherichia coli (LPS). As expected, plasma TC and TG concentrations were lower on the PUFA diet than on TRANS or SAFA diets. Aortic intima macrophage infiltration, ABCA1 content, and lesion area on PUFA group were lower compared to TRANS and SAFA groups. Macrophages and ABCA1 markers did not co-localize in the atherosclerotic plaque, suggesting that different cell types were responsible for the ABCA1 expression in plaques. Compared to PUFA, TRANS and SAFA presented higher collagen content and necrotic cores in atherosclerotic plaques. In the artery wall, TC was lower on PUFA compared to TRANS group; free cholesterol was lower on PUFA compared to TRANS and SAFA; cholesteryl ester concentration did not vary among the groups. Plasma TNF-α concentration on PUFA and TRANS-fed mice was higher compared to SAFA. No difference was observed in IL-6 concentration amongst groups. Regarding the macrophage inflammatory response to LPS, TRANS and PUFA presented higher culture medium concentrations of IL-6 and TNF-α as compared to SAFA. The PUFA group showed the lowest amount of the anti-inflammatory marker IL-10 compared to TRANS and SAFA groups. In conclusion, PUFA intake prevented atherogenesis, even in a pro-inflammatory condition.

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1. Introduction

It is well documented that the quantity and type of dietary fatty acids have an important role in the development of cardiovascular disease (CVD), especially due to their impact on plasma lipids, lipoprotein metabolism [1] and inflammatory status [2].

Polyunsaturated fatty acids have beneficial effects on cardiovascular risk [1], in part because of their hypolipidemic actions, although the underlying mechanisms are incompletely understood.

According to experimental studies [3,4] and clinical trials [1] strong association has been described between cardiovascular disease and saturated fatty acids and more pronounced with TRANS-fat consumption. Although saturated fatty acids have important intracellular functions [5], their high intake raises plasma low density lipoprotein-cholesterol (LDL-c) concentration, increasing cardiovascular risk. Moreover, trans fatty acids also induce a pro-atherogenic lipid profile and adversely decrease high density lipoprotein cholesterol (HDL-c) concentration [1].

The anti-atherogenic properties of HDL are, in part, attributed to its role in reverse cholesterol transport (RCT) [6], and several proteins and receptors involved in this process (e.g., CETP, LDLr, apoAI) are modulated by dietary fatty acids [7–9]. The most important transporters implicated in the cholesterol efflux from
An expanded methods section is provided in the Supplementary Material.

Animals and diet: Weaned male LDLr-KO mice with a C57BL/6 background were acquired from The Jackson Laboratory (Bar Harbor, ME, USA) and fed one of the three high-fat diets (40% of energy as fat) enriched with trans, saturated or polyunsaturated fatty acids did not interfere with the HDL-mediated cholesterol efflux in young adults [13]. These results suggest that the underlying mechanisms involved on the atherogenic capacity of trans and saturated fatty acids seem to be related to other steps of cholesterol transport, which deserve investigation.

The inflammatory process, which contributes to the development of atherosclerosis, is also modulated by dietary fatty acids [14]. Macrophages and foam cells in the arterial wall secrete pro-inflammatory factors such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-a) that increase local inflammation and contribute to lesion progression [15]. Both saturated fatty acids [16] and trans fatty acids are known to increase the secretion of pro-inflammatory markers [17] whereas polyunsaturated fatty acids, especially the n-3 series, can do the opposite [16].

Although the many actions of different types of dietary fatty acids on cardiovascular endpoint studies have been described, the consequences of the simultaneous impact of the inflammatory processes and plasma lipid metabolism on the development of atherosclerosis are not yet fully elucidated.

Therefore, this study was designed to evaluate the effects of high fat diets enriched with trans, saturated or polyunsaturated fatty acids on the concomitant impact of plasma lipids and the inflammatory response on the development of atherosclerotic lesions in LDLr-KO mice.

2. Materials and methods

<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Description</th>
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<tbody>
<tr>
<td>apoAI</td>
<td>apolipoprotein AI</td>
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<tr>
<td>apo B</td>
<td>apolipoprotein B</td>
</tr>
<tr>
<td>ABCA1</td>
<td>ATP binding cassette transporters A1</td>
</tr>
<tr>
<td>ABCG1</td>
<td>ATP binding cassette transporters G1</td>
</tr>
<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
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<tr>
<td>CETP</td>
<td>cholesteryl ester transfer protein</td>
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<tr>
<td>FPLC</td>
<td>fast protein liquid chromatography</td>
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<td>HDL</td>
<td>high density lipoprotein</td>
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<tr>
<td>LCAT</td>
<td>lecithin cholesterol acyl transferase</td>
</tr>
<tr>
<td>GLC</td>
<td>gas liquid chromatography analysis</td>
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<tr>
<td>LDLr</td>
<td>LDL receptor</td>
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<tr>
<td>LDLreceptor</td>
<td>low density lipoprotein</td>
</tr>
<tr>
<td>LDLr-KO</td>
<td>LDL receptor knockout</td>
</tr>
<tr>
<td>LCAT</td>
<td>lecithin cholesterol acyl transferase</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate-buffered saline</td>
</tr>
<tr>
<td>PUFA</td>
<td>diet enriched with polyunsaturated fatty acids</td>
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<tr>
<td>RCT</td>
<td>reverse cholesterol transport</td>
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<tr>
<td>SAFA</td>
<td>diet enriched with saturated fatty acids</td>
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<tr>
<td>TG</td>
<td>total cholesterol</td>
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<tr>
<td>TG</td>
<td>triacylglycerol</td>
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<tr>
<td>TRANS</td>
<td>diet enriched with trans fatty acid</td>
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<td>VLDL</td>
<td>very low density lipoprotein</td>
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 macrophages are the ATP binding cassette transporters A1 (ABCA1) and ABCG1 that work synergistically. ABCA1 transports phospholipids (PL) and free cholesterol (FC) onto apolipoprotein AI (apoAI), generating pre-β HDL, whereas ABCG1 promotes cholesterol efflux from macrophages onto mature HDL particles [10].

In vitro, polyunsaturated fatty acids undeniably diminish cellular ABCA1 content and, consequently, reduce the cell cholesterol and phospholipid apolipoprotein-mediated RCT [11,12]. Our previous data showed that diets enriched with trans, saturated or polyunsaturated fatty acids did not interfere with the HDL-mediated cholesterol efflux in young adults [13]. These results suggest that the underlying mechanisms involved on the atherogenic capacity of trans and saturated fatty acids seem to be related to other steps of cholesterol transport, which deserve investigation.
utilized for the atherosclerosis analysis so as to ensure the same arterial site. Briefly, sections were incubated overnight (4 °C) with primary antibodies recognizing ABCA1 (rabbit anti-mouse ABCA1, 1:500; Novus Biologicals, Littleton, CO, USA) or macrophages (rat anti-mouse CD68, 1:600; AbD Serotec, Raleigh, NC, USA), blocked for endogenous peroxidase, incubated with secondary antibodies conjugated with peroxidase (1:500; Abcam, MA, USA; and AbD Serotec, Raleigh, NC, USA) and detected with a DAB substrate kit (Vector Laboratories, CA, USA). The sections were counterstained with Carazzi’s Hematoxylin. The stained (brown) areas were measured utilizing Qwin image analysis software (Leica Imaging Systems, UK). The area was expressed as the mean area of the sections for 6–7 animals per group. A negative control was established by omitting the primary antibodies.

For confocal microscopy, cryostat sections (4 µm) of the aortic root were blocked and simultaneously incubated with polyclonal rabbit anti-mouse ABCA1 (1:50; Novus Biologicals) and rat anti-mouse CD68 (1:400, AbD Serotec) in a dark chamber for 90 min. After washing, AlexaFluor® 633-conjugated goat anti-rabbit IgG and AlexaFluor® 488-conjugated donkey anti-rat (Invitrogen) were utilized to detect ABCA1 and CD68, respectively, with a dual immunofluorescent staining. The nuclei were stained using DAPI (Sigma-Aldrich). Omission of the primary antibodies was used as the negative control. The sections were observed with a Zeiss LSM 510 Meta confocal laser-scanning microscope using the corresponding channel detector.

Peritoneal macrophage culture and stimulation: Peritoneal macrophages were harvested by peritoneal puncture, without adding any stimulant to induce macrophage migration to the peritoneal cavity, using sterile PBS, without EDTA, pH 7.4. Cells were cultured on a 24-mm multiwell plate in RPMI medium (Sigma-Aldrich) containing 10% BSA (Invitrogen), penicillin and streptomycin, and were maintained in a 5% CO₂ incubator at 37 °C. After this period the cells were washed remaining only adhered macrophages in the plaque. Cells (0.5 × 10⁶/well) were stimulated with 1 µg/mL lipopolysaccharide (LPS) from Escherichia coli (Sigma Chemical Co., St. Louis, MO) for 24 h, and inflammatory factors (TNF-α, IL-6 and IL-10) released into the culture medium were

Fig. 1. Dietary fatty acids and the development of atherosclerosis. The atherosclerotic lesion area was determined in the aortas isolated from LDLr-KO mice fed a high fat diet for 16 weeks. Serial sections were analyzed for (A) lipid content using Oil Red-O stain; (B) macrophage infiltrate (anti-CD68); and (C) ABCA1 content using immunohistochemistry (anti-ABCA1). The following parameters were quantified: (D) the atherosclerotic lesion area (n = 5); (E) macrophage infiltrate (n = 7) and (F) ABCA1 content (n = 7); (G) determination of the ABCA1: macrophage ratio of the atherosclerotic plaque; (H) aortic cholesterol content in whole aortas (n = 8–13) isolated from LDLr-KO mice fed either a TRANS, SAFA or PUFA diet for 16 weeks; *p < 0.05, **p < 0.001. Data were checked for normality and transformation was performed prior to statistical analysis. One-way ANOVA followed by the post-hoc Newman–Keuls Multiple Comparison Test for pair-wise comparisons was performed. Results are shown as the mean ± SEM.
measured using a commercial ELISA kit (R&D Systems, Minneapolis, MN).

**Statistical analysis:** The data were checked for normality and appropriate transformation were performed when necessary, prior to statistical analysis. One-way ANOVA followed by the post-hoc Newman–Keuls Multiple Comparison Test for pair-wise comparisons was performed. The correlations were calculated using a Spearman Test. A value of $p < 0.05$ was considered statistically significant. The data were analyzed with the GraphPad Prism software (GraphPad Software Inc., San Diego, CA). Untransformed data are presented in the figures, tables and text as the mean ± SD or SEM.

3. Results

The dietary intake did not differ among the groups. However, animals on the TRANS diet gained significantly less weight during the 16 weeks period than the other groups (weight gain (g): TRANS: 17.17 ± 1.86, SAFA: 19.26 ± 3.24 and PUFA: 20.44 ± 3.46; $p < 0.05$).

3.1. Dietary fatty acids and the development of atherosclerosis

PUFA-fed mice presented the lowest amount of lipids in the arterial wall, intima macrophage infiltration and ABCA1 content compared to the SAFA and TRANS-fed mice (Fig. 1A–F).

The atherosclerotic plaque ABCA1: macrophage ratio for the PUFA-fed mice was lower than in the TRANS-fed mice but did not differ from the SAFA-fed mice (Fig. 1G).

Regarding the cholesterol content of the artery wall, TC was lower with PUFA feeding than with TRANS but did not differ from the SAFA feeding. Free cholesterol (FC) was lower in PUFA-fed mice compared to TRANS and SAFA groups; cholesteryl ester (CE) did not vary among the groups (Fig. 1H). The TC on the aorta was positively correlated with lesion area ($r = 0.56$, $p = 0.037$), plasma TC ($r = 0.47$, $p = 0.005$), and plasma TG ($r = 0.52$, $p = 0.002$); lesion area was positively correlated with CE and FC of the total aorta ($r = 0.73$, $p = 0.002$ and $r = 0.66$, $p = 0.01$, respectively) (Fig. 2).

The liver expression of ABCA1 has a primordial role in HDL biogenesis [21] and consequently on the prevention of atherosclerosis [22]. However, in the present study, the intake of diets enriched with TRANS, SAFA or PUFA had similar effects on ABCA1 mRNA expression in the livers of the LDLr-KO mice (Supplemental Fig. 1).

3.2. Localization of ABCA1 and macrophages on the atherosclerotic plaque

Immunohistochemical analyses suggested that ABCA1 and macrophage expression were found in the same region of the atherosclerotic plaque (Supplemental Fig. 2). However, the dual immunofluorescent staining (Fig. 3), in serial sections, did not show co-localization of macrophages and ABCA1 in the plaques. This suggested that although some expression of ABCA1 can be attributed to macrophages (CD68 positive cells), other cells types are likely responsible for the majority of the ABCA1 expression in the plaque.

3.3. Plasma lipids and lipoprotein profile

The PUFA group had the lowest plasma TC and TG concentration compared to TRANS- and SAFA-fed mice. TRANS-fed mice developed severe hypercholesterolemia and hypertriglyceridemia (Table 1). A greater atherogenic lipid profile was observed in the TRANS group because of the higher amount of cholesterol transported in non-HDL particles (94%), with 67% in VLDL and only 6% in HDL (Table 1). Moreover, the TRANS diet induced a higher VLDL-TG
concentration compared to the SAFA and PUFA diets and a higher HDL-TG concentration when compared to the PUFA diet.

Plasma TC and TG concentrations were positively correlated with the arterial macrophage infiltration (TC: \(r = 0.69, p = 0.0004\); TG: \(r = 0.63, p = 0.001\), ABCA1 content (TC: \(r = 0.70, p = 0.0002\); TG: \(r = 0.61, p = 0.002\)) and atherosclerotic lesion area (TC: \(r = 0.77, p = 0.001\); TG: \(r = 0.86, p = 0.0001\)). These results support the predominant role of plasma cholesterol on the development of atherosclerotic lesions associated with the different diets (Fig. 4).

Table 1
Plasma total cholesterol (TC), triglycerides (TG) concentrations (mmol/L) are listed, and the lipoprotein profiles are expressed as absolute concentration (mmol/L) and percent distributions. Data are from LDLr-KO mice fed a TRANS, SAFA or PUFA diet for 16 weeks.

<table>
<thead>
<tr>
<th></th>
<th>TRANS</th>
<th>SAFA</th>
<th>PUFA</th>
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<tbody>
<tr>
<td>TC (mmol/L)</td>
<td>27.17 ± 1.10a</td>
<td>10.39 ± 3.29b</td>
<td>4.92 ± 2.10c</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>4.17 ± 1.40a</td>
<td>1.81 ± 0.55b</td>
<td>1.11 ± 0.57c</td>
</tr>
<tr>
<td>VLDL-C (mmol/L)</td>
<td>18.16 ± 7.56a</td>
<td>4.40 ± 3.13b</td>
<td>1.27 ± 0.65c</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>7.59 ± 3.99a</td>
<td>4.14 ± 1.97b</td>
<td>2.36 ± 1.40b</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.40 ± 0.26a</td>
<td>1.81 ± 0.70b</td>
<td>1.30 ± 0.70b</td>
</tr>
<tr>
<td>non-HDL-C (mmol/L)</td>
<td>25.77 ± 11.03a</td>
<td>8.55 ± 3.55b</td>
<td>3.63 ± 1.45b</td>
</tr>
<tr>
<td>VLDL-TG (mmol/L)</td>
<td>3.34 ± 1.27a</td>
<td>1.29 ± 0.41b</td>
<td>0.82 ± 0.45b</td>
</tr>
<tr>
<td>LDL-TG (mmol/L)</td>
<td>0.90 ± 0.18a</td>
<td>0.31 ± 0.12b</td>
<td>0.24 ± 0.12b</td>
</tr>
<tr>
<td>HDL-TG (mmol/L)</td>
<td>0.33 ± 0.27a</td>
<td>0.19 ± 0.24b</td>
<td>0.05 ± 0.03b</td>
</tr>
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3.4. Plasma cytokines and the inflammatory response

No difference was observed in IL-6 concentration amongst the groups (Fig. 5A). Plasma TNF-\(\alpha\) concentration on PUFA- and TRANS-fed mice was higher compared to SAFA-fed mice after the 16-week intake period (Fig. 5B). Regarding the macrophage inflammatory response to LPS, PUFA- and TRANS-fed mice presented higher concentrations of IL-6 and TNF-\(\alpha\) compared to SAFA, confirming the plasma findings. Compared to TRANS and SAFA groups, the PUFA group showed the lowest amount of the anti-inflammatory marker IL-10. The PUFA diet was rich in \(\omega\)-6 fatty acids, which may explain the pro-inflammatory response observed (Fig. 5C).

3.5. Vulnerability of the atherosclerotic plaque

TRANS- and SAFA-fed mice presented a higher amount of collagen on the atherosclerotic plaques compared to PUFA-fed mice, however, as expected, in this group the collagen content was nearly undetectable, because they presented the lowest degree of atherosclerosis. Moreover, the atherosclerotic lesions in mice fed a TRANS or a SAFA diet had a thin fibrous cap with a necrotic core characterizing unstable plaques (Fig. 6).

4. Discussion

This study demonstrates for the first time that the intake of a high-fat diet enriched with \(\omega\)-6 polyunsaturated fatty acids prevented the development of atherosclerotic lesions even in a pro-inflammatory condition. However, though showing a similar effect on the inflammatory profile, TRANS-fat led to severe atherogenesis. These results reinforce the preponderance of plasma lipid concentration on the atherosclerotic development [23]. The PUFA...
Fig. 4. Correlations between plasma lipid concentrations (TC and TG) and aortic root ABCA1, and macrophage infiltration and lesion areas. Plasma TC and TG concentrations were positively correlated with aortic ABCA1 content, macrophage infiltration and, atherosclerotic lesion area.

Fig. 5. Plasma inflammatory profile and macrophage inflammatory response. Peritoneal macrophages isolated from mice fed one of the three fatty diets for 16 weeks, and cultured in RPMI medium for 48 h. After this period the cells were washed remaining only adhered macrophages in the plaque. Cells (0.5 × 10^6/well) were stimulated with 1 μg/mL lipopolysaccharide (LPS) from Escherichia coli, for 24 h, and inflammatory factors (TNF-α, IL-6 and IL-10) released into the culture medium were measured using a commercial ELISA kit: (A) plasma IL-6 (n = 4); (B) plasma TNF-α (n = 4); (C) culture medium cytokines (n = 4).
group presented a higher plasma TNF-α concentration as compared to SAFA-fed mice, but similar to TRANS group. In accordance to plasma findings, peritoneal macrophages from animals fed PUFA and TRANS diets elicited higher secretions of pro-inflammatory cytokines (IL-6 and TNF-α) in the medium compared to animals on a SAFA diet. The anti-inflammatory mediator, IL-10, was lower in the PUFA group compared to TRANS and SAFA groups. Even with an inflammatory response similar to the TRANS group, the PUFA group presented the lowest degree of atherosclerosis, arterial lipid content, macrophage infiltration and ABCA1 content as compared to TRANS and SAFA groups.

This study was performed in LDLr-KO mice which are moderately hypercholesterolemic and widely used to investigate the development of atherosclerosis. When submitted to a high fat diet, these animals become hypercholesterolemic and develop atherosclerosis, even in the absence of cholesterol in the diet. In a recent study [24], the plasma cholesterol concentration observed in LDLr-KO mice, submitted to a high cholesterol diet was similar to our results in TRANS fed-mice. This indicates that a high fat diet is sufficient to elevate plasma cholesterol concentration and bring about atherosclerotic lesions. In another study [25], mice submitted to a normal cholesterol high fat diet developed a more severe hypercholesterolemia and larger lesions in the aortic origin cross sections than those submitted to a normal fat high cholesterol diet. Moreover the addition of cholesterol to the diet would blunt the biological effect of each fatty acid on the development of atherosclerosis.

Our data corroborate the results of epidemiological trials in which the cardiovascular endpoint benefits of polyunsaturated fatty acids are demonstrated [26,27]. These data reinforce the notion that some unfavorable actions of these fatty acids should be cautiously interpreted. Regarding inflammation, ω-6 polyunsaturated fatty acids are known for their pro-inflammatory capacity [28]. Arachidonic acid is a substrate for eicosanoid synthesis which is enrolled on the control of intensity and duration of inflammatory responses [2]. Dietary fatty acids are incorporated into the plasma membranes including on inflammatory cells, and therefore can modulate their responses [2].

Despite the pro-inflammatory effect, the PUFA group presented the lowest plasma TG and TC concentrations compared to the TRANS and SAFA groups. Moreover, in the PUFA group 25% of total plasma cholesterol is carried by HDL, compared with 19% on SAFA and 6% on TRANS group, as shown in the lipid profile (Table 1). This suggests that HDL is efficiently delivering the cholesterol to the liver so it can be excreted in to the bile. The improvement in the lipid profile due to PUFA administration supplanted the detrimental effect that inflammation might have caused.

Dietary fatty acids modulate the composition of cell membrane, which resembles the fatty acids on the diet. This composition will result in a difference in membrane fluidity that can interfere on proteins activity and cell signalization. Another effect of dietary fatty acids is related to Toll-Like Receptors (TLR). TLR 4, for example, binds lipopolysaccharide (LPS) and can also recognize molecules that are not derived from bacteria, such as heat shock protein 60, fibronectin, fibrinogen and certain types of fatty acids. Saturated fatty acids are able to induce secretion of inflammatory markers in a manner analogous to LPS [16]. Animals and humans can develop endotoxin tolerance to LPS after chronic exposition [29]. Similarly,
saturated fatty acids might have stimulated TLR4 during the feeding period, and thus peritoneal macrophages when exposed to LPS treatment have a lower pro-inflammatory response. At this time, this hypothesis cannot be supported by our data. Another interesting point that should be further investigated is the presence of T cells and cytokines in the plaque, which would give a better characterization of the inflammatory status in the lesion. Unfortunately, we did not have sufficient material to answer this point. The TRANS diet induced severe hypertriglyceridemia and hypercholesterolemia, probably as a result of a higher synthesis and secretion of apoB-lipoproteins in this group. It has been shown that trans fatty acids increase the liver lipogenic capacity [18]. Moreover, the TRANS diet presented a pro-atherogenic profile, as confirmed in other studies [1,3] with ApoB particles largely enriched with cholesterol. Like all industrialized hydrogenated fats, the TRANS diet was also rich in stearic acid. However, the results obtained in this study group can be attributed to the trans fatty acid content because in the liver, stearic fatty acid is rapidly converted to oleic acid by the action of stearoyl-CoA desaturase 1 (SCD1) [30].

Hepatic ABCA1 content has a major impact on the development of atherosclerosis due to its role in HDL formation; therefore, the reduction of hepatic ABCA1 can contribute to lesion progression. It has been demonstrated that unsaturated fatty acids lowered the ABCA1 protein level by increasing its degradation rate, an action that has been noted by some authors to be undesirable [11,12]. Although the biological function of this down-regulation remains unclear, several clinical and epidemiological studies have highlighted the beneficial effects of unsaturated fatty acid consumption [26,27]. In the present study, liver ABCA1 mRNA expression did not differ among the groups, suggesting that the dietary fatty acids exerted similar modulation on the ABCA1 transcription level. According to the present study, whichever mechanism is involved in the increased degradation of ABCA1 by polyunsaturated fatty acids in the in vitro studies, it does not impact in cardiovascular risk.

A well-designed study, performed in mouse macrophage or human monocyte-derived macrophage, demonstrated that elaidic acid reduced the ABCA1-mediated cholesterol efflux, likewise palmitic acid [31]. This effect was not at the ABCA1 transcription, stability or protein level, once the ABCA1 mRNA expression and cell-surface protein content were not altered. Moreover, the authors suggest that these results are likely due to a decrease of membrane fluidity [31]. These results help to explain why, despite the large ABCA1 content on the atherosclerotic lesion, TRANS and SAT groups were not able to prevent the lesion progression.

Another novel finding presented here is that ABCA1 and macrophages did not co-localize in the atherosclerotic lesion, as clearly demonstrated by confocal microscopy. These results indicate that besides macrophages, different cell types appear to be involved in ABCA1 plaque expression. Smooth muscle cells (SMC) migrate from the media into the intimal layer in response to cytokines and inflammatory mediators, which also induce their proliferation and change from a quiescent contractile state to an active synthetic condition [32]. Under a cholesterol loading condition, SMCs are converted to macrophage-like cells and acquire macrophage function by increasing the expression of macrophage-related genes and by losing markers of the SMC phenotype [33]. Intima phenotype-SMCs present lower ABCA1 expression, apoA1 binding, HDL formation and also an impaired apoA1-mediated cholesterol efflux [34]. We are currently investigating the contribution of SMCs on the atherosclerosis development and its ABCA1 expression. Although this answer might be very important, at this point these results would not modify our conclusions.

In the current study, the collagen content of the PUFA-fed mice was barely discernible. The TRANS- and SAFAs-fed mice developed atheroma with a thin fibrous cap shielding a necrotic core, characterizing unstable plaques that are more prone to disruption and frequently precede thrombosis-mediated events [35].

It has been shown that ABCA1 expression in macrophages located in the lesion area may exert a secondary role against the progression of atherosclerosis when compared to the severity of hyperlipidemia [22]. Our results corroborate these findings, and additionally, we demonstrated that polyunsaturated fatty acids diminished the ABCA1 content in the atherosclerotic lesion. Moreover, in the PUFAs group, this effect has been associated with an inflammatory profile and the lowest CT and TG concentrations that culminate in atherosclerotic prevention. However, despite a pro-inflammatory status similar to PUFAs feeding, trans fatty acid consumption induces severe hyperlipidemia, which had a remarkable atherogenic impact.

In conclusion, this work reinforces the “Lipid Hypothesis” [23], where the plasma cholesterol plays a crucial role in the development of atherosclerosis even in the presence of an inflammatory profile as observed in the PUFAs group.

Acknowledgments

The authors’ responsibilities were as follows: RMM and AMPL contributed to the study concept and design, conducted the experiments, data analyses, interpretation of the results and writing of the manuscript; ERN and ECRQ contributed to the study design, interpretation of the results and the writing of the manuscript; PMcC, MKK, VSN, DFD, MSA, RPAB, AML and FGS conducted the experiments and data analyses; SC contributed by providing animals. All authors reviewed the final manuscript, and none of the authors had a financial or personal conflict of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.atherosclerosis.2012.06.059.

References


