Pomegranate Juice Protects Macrophages from Triglyceride Accumulation: Inhibitory Effect on DGAT1 Activity and on Triglyceride Biosynthesis

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Key Words
Macrophages · Pomegranate juice · Punicalagin · Triglyceride · Diacylglycerol acyltransferase 1 · Paraoxonase 2

Abstract
Background/Aims: To analyze the effects of pomegranate juice (PJ) and punicalagin on macrophage triglyceride metabolism. Methods: Triglyceride metabolism was analyzed in PJ- or punicalagin-treated J774A.1 macrophages or in mouse peritoneal macrophages (MPM) harvested from C57BL/6 mice or from paraoxonase 2 (PON2)-deficient mice. Results: PJ (0–50 μM) significantly and dose-dependently decreased the triglyceride content and triglyceride biosynthesis rate in J774A.1 macrophages or in C57BL/6 MPM by about 30%. Similarly, punicalagin, the major PJ polyphenol, inhibited the MPM triglyceride biosynthesis rate by 40%. The triglyceride hydrolytic rate, however, was not significantly affected by PJ or punicalagin. The activity of diacylglycerol acyltransferase 1 (DGAT1; the rate-limiting enzyme in triglyceride biosynthesis) was significantly inhibited, by 54%, in C57BL/6 MPM that were treated with 50 μM PJ or punicalagin, with no significant effect on DGAT1 mRNA or protein expression. Both PJ and punicalagin increased (1.7-fold) MPM PON2 mRNA expression, and PON2 was previously shown to inhibit DGAT1 activity. However, the addition of PJ or punicalagin (50 μM) to microsomes from PON2-deficient MPM still resulted in a significant reduction (50–58%) in DGAT1 activity. Conclusions: We conclude that the inhibitory effect of PJ on triglyceride biosynthesis could be attributed to a direct effect of PJ on DGAT1 activity.

Introduction

Triglycerides are an independent risk factor for atherosclerosis [1, 2]. Macrophage lipid accumulation and foam cell formation is the hallmark of early atherogenesis [3–5]. Indeed, atherosclerotic lesions and macrophage foam cells isolated from these lesions contain predominantly cholesterol esters and a substantial amount of triglycerides [6–8]. Triglyceride accumulation in macrophages increased oxidative stress and cellular necrosis [9], thus further contributing to foam cell formation.

Pomegranate juice (PJ) was shown to be a most potent antioxidant, and this property was shown to be due to its polyphenolic ellagitannin compounds such as punicalagin [10, 11]. PJ is a heart-healthy fruit juice [12]. Indeed, previous studies have demonstrated that PJ consumption by healthy volunteers significantly decreased LDL and HDL oxidation [13]. Moreover, consumption of PJ by patients with carotid artery stenosis significantly increased serum paraoxonase 1 (PON1) activity and decreased serum oxidative stress, lesion oxidative stress, and lesion...
Table 1. PJ antiatherogenic effects: in vitro and in vivo studies

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HDL = High-density lipoprotein.

... cholesterol content; it also inhibited atherosclerotic plaque development in the carotid arteries [14, 15]. Similarly, consumption of PJ by diabetic patients significantly decreased serum oxidative stress and the extent of oxidized LDL (Ox-LDL) uptake by their macrophages [16]. These effects could be related to a PJ-induced increment in PON1 binding to the HDL and in its catalytic activities [17, 18]. Mice studies have also demonstrated the beneficial antiatherogenic effects of PJ. Consumption of PJ by atherosclerotic apolipoprotein E-deficient mice resulted in an increment in serum PON1 activity, a reduction in serum and macrophage oxidative stress, a decrement in the extent of macrophage Ox-LDL uptake, and an increment in HDL-mediated cholesterol efflux from the cells. All of these effects lead to the attenuation of atherosclerosis development in these mice [13, 19, 20]. In vitro studies, using the J774A.1 macrophage cell line, revealed that PJ decreases cellular oxidative stress, inhibits the macrophage cholesterol biosynthesis rate and the extent of Ox-LDL uptake by macrophages, and increases the extent of cholesterol efflux from the cells by HDL [13, 20, 21].

Mammalian paraoxonases (PON1, PON2, and PON3) are a unique family of calcium-dependent esterases/lactonases [22] with enzymatic activities towards a broad range of substrates. Whereas PON1 is expressed mainly in the liver and is present in circulation, PON2 is expressed in most tissues, including macrophages [23, 24]. PON2, like PON1, was shown to protect against atherosclerosis development [25]. This effect could be attributed to a PON2-induced reduction in macrophage oxidative stress and in triglyceride accumulation [23, 26, 27]. PJ was shown to increase PON2 expression in J774A.1 macrophages [28]. Upon using PON1-deficient mice, or PON2-deficient mice in comparison to C57BL/6 mice that consumed PJ, we recently demonstrated that the antioxidant properties of PJ consumption in mouse macrophages, but not the beneficial effects of PJ on macrophage cholesterol and triglyceride metabolism, are mediated via the PJ-induced stimulation of macrophage PON2 [29]. However, the data concerning the effect of PJ on triglyceride metabolism in this study is minimal.

Thus, in the present study we analyzed in vitro the possible role of PJ in protecting macrophages from triglyceride accumulation and its mechanism of action. Furthermore, we questioned whether the inhibitory effect of PJ is a direct effect of PJ and/or is mediated by macrophage PON2.

Table 1 summarizes the antiatherogenic effects of PJ both in vitro and in vivo.

Materials and Methods

PJ and Punicalagin

PJ was obtained from PomWonderful, Los Angeles, Calif., USA, and punicalagin was a generous gift from Dr. Daneel Ferrara, University of Mississippi, USA.

Pomegranates were picked by hand, washed, and stored in tanks. The fruit was crushed and squeezed. The juice was filtered, pasteurized, concentrated, and stored at –18°C. The PJ total phenolics concentration measured in gallic acid equivalents was 3,600 mg/l (19 mM), including mainly hydrolyzable tannins (ellagitannins), such as oligomers and punicalagin/punicalin, with smaller amounts of ellagic acid and anthocyanins (delphinidin, cyanidin, and pelargonidin) and their glycosides. PJ contains 1,740 mg/l of punicalagin. Other components of PJ are: sugars, 13.8%; organic acids, 0.6%; ash, 0.5%; potassium, 0.21%, and protein, 0.1%.

Punicalagin was dissolved in phosphate buffer solution (PBS) to a stock solution of 1 mg/ml. In the current study we used total polyphenol concentrations of 0–50 μM PJ or punicalagin. These concentrations were not cytotoxic to the cells as determined by a cell viability test and by lactate dehydrogenase release into the incubation medium.
**PON2-Deficient Mice**

The PON2-deficient mice on the C57BL/6 background were generated as previously described [25]. The mice (males) were fed with a chow diet. The research was conducted in conformity with the Public Health Service Policy on Human Care and Use of Laboratory Animals. The studies were approved by the Committee for Supervision of Animal Experiments, Technion – Israel Institute of Technology, Haifa, Israel (No. ILO46-04-2008). PON2-deficient mice and C57BL/6 mice were sacrificed at the age of 4 months and their peritoneal macrophages were harvested.

**Cells**

J774 A.1 murine macrophage cells were purchased from the American Tissue Culture Collection (ATCC; Rockville, Md., USA). The cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) containing 5% fetal calf serum (FCS).

Mouse peritoneal macrophages (MPM) were harvested from the peritoneal fluid of 10 control (C57BL/6) mice or from 10 PON2-deficient mice 4 days after intraperitoneal injection into each mouse of 3 ml of aged thioglycolate (40 g/l) in saline. The cells (10–20 × 10^6/mouse) were washed and centrifuged 3 times with PBS at 1,000 g for 10 min and then resuspended at 10^6/l in DMEM containing 5% FCS, 1 × 10^5 units penicillin/l, 100 mg/l streptomycin, and 2 mmol/l glutamine. The dishes were incubated in a humidified incubator (5% CO₂ and 5% air) for 2 h and then washed with DMEM to remove nonadherent cells, and the analyses to measure cellular oxidative stress were immediately performed.

**Cell Incubation with PJ or Punicalagin**

The cells were incubated with increasing concentrations of PJ or punicalagin (0–50 μM) for 20 h at 37°C in DMEM + 5% FCS.

**MPM Triglyceride Mass**

The macrophage lipids were extracted with hexane:isopropanol (3:2, v:v), and the hexane phase was evaporated under nitrogen. The amount of cellular triglycerides in the dried samples was determined (after addition of 50 μl DMSO) using a triglyceride determination kit (Sigma, catalog No. TR0100). The remaining cells in the dish were dissolved in 0.1 M NaOH, and an aliquot was taken for the measurement of cellular protein by Lowry assay [30].

**Separation of Cellular Lipids by Thin-Layer Chromatography**

The macrophage lipids were extracted with hexane:isopropanol (3:2, v:v) and the hexane phase was evaporated under nitrogen. Chloroform was added to the dried samples, followed by their loading on silica gel 60 F254 plates (20 × 20 cm; Merck, Darmstadt, Germany) and lipid separation using a mixture of 130 ml hexane, 30 ml ether, and 1.5 ml acetic acid. The separated lipids were visualized using iodine vapor.

**MPM Triglyceride Biosynthesis Rate**

The amount of [3H]-oleate incorporated into triglycerides was determined as previously described [31]. Cells were incubated for 3 h at 37°C in serum-free medium containing 6 μCi/ml [3H]-oleate bound to fatty acid-free bovine serum albumin (BSA; 2%). After incubation, the cells were washed twice with PBS, pH 7.4; the lipids were extracted and separated by thin-layer chromatography (TLC), and the radioactivity in the bands corresponding to triglycerides was determined by liquid scintillation counting. The remaining cells in the dish were dissolved with 0.1 M NaOH, and an aliquot was taken for the measurement of cellular protein [30].

**MPM Microsome Separation**

Microsomes were isolated from MPM of PON2-deficient or C57BL/6 mice as previously described [32]. The cells (20 × 10^6) were washed with cold PBS and scraped from the dishes into buffer A (50 mM Tris/HCL, pH 7.4, 250 mM sucrose, and mix protease inhibitors) following sonication for 3 × 10 s, resulting in about 95% of cell breakage. Nuclei, granules, and unbroken cells were removed by 2 centrifugation steps: the first one for 15 min at 1,200 g and 4°C and the second one for 15 min at 9,000 g. The supernatant obtained after the second centrifugation was collected and further centrifuged at 100,000 g to obtain the microsomal pellet. The pellet was suspended in buffer A, and the protein concentration was determined by Bradford assay.

**DGAT1 and PON2 mRNA Expression by Reverse Transcriptase Quantitative Polymerase Chain Reaction**

RNA was extracted from the cells using a MasterPure™ RNA purification kit (Epicentech Biotechnologies, Madison, Wisc., USA). cDNA was prepared using a Verso™ cDNA kit (Thermo Scientific, Epsom, UK). Primers and probes for DGAT1, PON2, and GAPDH were designed by Primer Design, Southampton, UK. Using Absolute Blue QPCR ROX mix (Thermo Scientific), expression was determined by quantitative real-time PCR with a Rotor-Gene 6000 amplification detection system.

**DGAT1 Protein Determination by Western Blot Analysis**

Western blot analysis was performed using SDS-PAGE and 10% bis-acrylamide gels. Cell lysates (20 μg protein/ml) were loaded onto the gel. Blocking of the gel was performed with 2% BSA for 2 h at room temperature. The primary antibody was goat polyclonal anti-human DGAT1 from Novous Biologicals [diluted 1:500 to a final concentration of 1 μg/ml, v/v, in TBS-T (5 mM NaCl, 2 mM Tris, pH 7.5, and Tween 20) with 0.5% BSA], and it was incubated with the nitrocellulose membrane at 4°C overnight. The secondary antibody, rabbit anti-goat horseradish-peroxidase-conjugated (Sigma-Aldrich), was diluted 1:10,000 in TBS-T and incubated for 1 h at room temperature. The membranes were developed using an EZ-ECL chemiluminescence detection kit (Biological Industries, Israel).

**Microsomal DGAT1 Activity**

DGAT1 activity was determined using microsomes (30 μg protein/ml), with a total volume of 200 μl, using reaction buffer (175 mM Tris-HCL, pH 8.0, 8 mM MgCl₂, and 5 mg/ml fatty acid-free BSA) supplemented with 1.5 nmol of 1,2-dioleoyl-glycerol (Sigma) and 1 nmol of [14C]-labeled oleoyl coenzyme A (Perkin Elmer, Boston, Mass., USA) [27]. The samples were incubated for 3 h at 37°C followed by lipid extraction and triglyceride separation by TLC. The radioactivity in the triglyceride spots was then counted.

**Statistical Analyses**

Each experiment was performed in triplicate, and each individual experiment was replicated 3 times (n = 3) in order to achieve statistical significance. Statistical analyses were performed using Student’s t test to compare differences between 2
groups and 1-way ANOVA, followed by the Student-Newman-Keuls test, to compare differences between multiple groups. Results are given as means ± SD.

**Results**

The Effect of PJ on Cellular Triglyceride Content and on the Rate of Triglyceride Biosynthesis in J774A.1 Macrophages and in MPM from C57BL/6 Mice

Incubation of the J774A.1 macrophage cell line (a, c) or MPM (b, d) were incubated for 20 h at 37 °C with a total polyphenol concentration (0–50 μM) of PJ. The cellular triglyceride content (a, b) and the macrophage triglyceride biosynthesis rate (c, d) were determined as described in Methods. Results are expressed as the mean ± SD of 3 different experiments. * p < 0.01 vs. concentration 0.

Fig. 1. Effect of PJ on cellular triglyceride mass and on the triglyceride biosynthesis rate: concentration studies in J774A.1 macrophages and in C57BL/6 MPM. The J774A.1 macrophage cell line (a, c) or MPM (b, d) were incubated for 20 h at 37 °C with a total polyphenol concentration (0–50 μM) of PJ. The cellular triglyceride content (a, b) and the macrophage triglyceride biosynthesis rate (c, d) were determined as described in Methods. Results are expressed as the mean ± SD of 3 different experiments. * p < 0.01 vs. concentration 0.

up to 27% (fig. 1a). Similarly, in C57BL/6 MPM, the cellular triglyceride content was significantly decreased by up to 30% upon incubating the cells with PJ (0–50 μM; fig. 1b). We next questioned whether the PJ-induced decrement in the macrophage triglyceride content results from inhibition of the triglyceride biosynthesis rate. Indeed, the rate of triglyceride biosynthesis in J774A.1 macrophages (fig. 1c) or in C57BL/6 MPM (fig. 1d) that were incubated with PJ (0–50 μM) was significantly reduced by up to 33 or 31%, respectively.
The Effect of PJ or Punicalagin on the MPM Triglyceride Biosynthesis Rate and on the MPM Triglyceride Hydrolysis Rate

Since punicalagin is the major ellagitannin polyphenol in PJ [10, 11], we next analyzed the effect of punicalagin in comparison to PJ on the macrophage triglyceride metabolism. Similarly to PJ, punicalagin (50 μM) inhibited the triglyceride biosynthesis rate in C57BL/6 MPM by 40% (fig. 2a). As the lower levels of triglycerides in PJ- or punicalagin-treated MPM could also possibly have resulted from an enhanced rate of triglyceride breakdown by specific lipases [33], we preincubated the cells with [3H]-oleic acid followed by a cell wash and further incubation for up to 4 h. After 4 h, the triglyceride hydrolysis rates in the PJ- or punicalagin-treated MPM were similar to those observed in the control nontreated cells (fig. 2b) as the labeled triglyceride level was decreased by 45, 39, or 41% in control cells, PJ-treated cells, or punicalagin-treated cells, respectively (fig. 2b). These observations indicate that the lower levels of triglycerides in PJ- or punicalagin-treated MPM are not the result of an enhanced rate of triglyceride breakdown but are rather the result of their inhibitory effect on the triglyceride biosynthesis rate.

The Effect of PJ or Punicalagin on Diacylglycerol Acyltransferase 1 Expression in MPM

Diacylglycerol acyltransferase 1 (DGAT1) is an endoplasmic reticulum membrane-associated enzyme that catalyses the final step in triglyceride biosynthesis by covalently attaching a long-chain fatty acyl-CoA to diacylglycerol. DGAT1 is expressed in all tissues including macrophages [34, 35]. In order to examine whether the decrease in the rate of macrophage triglyceride biosynthesis in PJ- or punicalagin-treated MPM is related to the inactivation of DGAT1, we determined microsomal DGAT1 activity. Microsomal DGAT1 activity was significantly inhibited, i.e. by 25 or 54%, in MPM that were incubated with 25 or 50 μM PJ, respectively (fig. 3a), as compared to DGAT1 activity in control (nontreated) cells. Similarly to the effect of PJ, DGAT1 activity was lower by 29 or 54% in punicalagin-treated cells (25 or 50 μM, respectively) (fig. 3a). This phenomenon could possibly be the result of posttranscriptional or posttranslational changes. Therefore, we also determined DGAT1 mRNA levels (as expressed by the DGAT1 mRNA/GAPDH mRNA ratio) by real-time PCR (fig. 3b) and DGAT1 protein levels (as expressed by the DGAT1/β-actin ratio) by Western blot analysis (fig. 3c). In PJ- or punicalagin-treated MPM versus control MPM, similar levels of DGAT1 mRNA or DGAT1 protein were observed, suggesting that PJ or punicalagin directly regulates DGAT1 at the activity level.

The Effect of PJ and Punicalagin on Triglyceride Accumulation in MPM from PON2-Deficient Mice

PON2, which is present in macrophages, was shown to inhibit triglyceride biosynthesis via a direct inhibitory effect on macrophage DGAT1 activity [26, 27]. PJ or punicalagin were shown to upregulate PON2 expression in J774A.1 macrophages [28] and, similarly to J774A.1 macrophages, we observed in MPM that PJ or punicalagin (50 μM) significantly increased PON2 mRNA levels up to 1.7-
fold from 0.44 ± 0.02 PON2 mRNA/GAPDH mRNA in control nontreated cells to 0.78 ± 0.01 or 0.71 ± 0.02 in PJ- or punicalagin-treated cells, respectively.

In order to examine whether the inhibitory effect of PJ on macrophage triglyceride biosynthesis is a direct effect of PJ constituents, we next used MPM from PON2-deficient mice (fig. 4). Incubation of the cells with increasing concentrations (0–50 μM) of PJ resulted in a significant reduction in cellular triglyceride content of up to 52% (fig. 4a). In accordance with these results, the MPM triglyceride biosynthesis rate was significantly decreased in PJ-treated cells by up to 39% (fig. 4b). These results indicate that PJ directly inhibits the macrophage triglyceride biosynthesis rate. Indeed, direct addition of PJ (50 μM) to microsomes obtained from PON2-deficient mice significantly inhibited DGAT1 activity by 50% (fig. 4c). Similarly, addition of punicalagin (50 μM) to MPM from PON2-deficient mice decreased the cellular triglyceride content by 60% (from 39.0 ± 1.0 to 15.6 ± 1.7 μg/mg cell protein), the triglyceride biosynthesis rate by 45% (from 83,306 ± 10,589 to 45,818 ± 4,600 cpm/mg cell protein), and microsomal DGAT1 activity by 58% (from 16,080 ± 220 to 6,754 ± 50 cpm/mg protein).

**Discussion**

The present study demonstrated, for the first time, the ability of PJ or punicalagin to attenuate macrophage triglyceride accumulation, secondary to the inhibition of triglyceride biosynthesis by DGAT1. These effects could be attributed directly to PJ constituents.

Macrophage cholesterol and triglyceride accumulation and foam cell formation are the hallmark of early atherogenesis [3–5]. In our study, we used several macrophage systems: the J774A.1 murine macrophage cell line and MPM harvested from C57BL/6 mice or from PON2-deficient mice. MPM resemble arterial macrophages in their morphology and biological activities [36].

In macrophages treated with PJ, the cellular triglyceride content was significantly lower than in control nontreated cells, and this effect was PJ dose dependent. These effects of PJ could be attributed, at least in part, to punicalagin. The macrophage triglyceride level at each time point is determined by the balance between the rate of triglyceride biosynthesis and the rate of their hydrolysis. However, whereas the synthesis rate of triglycerides was significantly inhibited in PJ- or punicalagin-treated macrophages versus control macrophages, the triglyceride hydrolytic rate (determined as the percent decrement in labeled triglyceride levels after 4 h) was similar in control MPM and in PJ- or punicalagin-treated MPM, indicating that neither PJ nor punicalagin decreased triglyceride hydrolysis.
punicalagin has any effect on these lipases. DGAT1, which is expressed in most tissues, including macrophages [34, 35, 37], is the rate-limiting enzyme in triglyceride biosynthesis which catalyzes the last step in the de novo triglyceride synthetic pathway, producing triglycerides from diacylglycerol and fatty acyl-CoA [34, 35, 38, 39]. Cellular triglyceride storage is directly correlated with the levels of DGAT1 activity. In PJ- or punicalagin-treated MPM versus control MPM, microsomal DGAT1 activity was significantly lower and this could have accounted for the observed inhibition of the triglyceride biosynthesis rate. Previous studies have indicated that DGAT1 is predominantly regulated posttranscriptionally [38–41]. The results of the present study also indicate that PJ or punicalagin regulate DGAT1 posttranscriptionally at the activity level. Similarly to the results observed in the current study, it has been shown that quercetin inhibits DGAT1 activity in CaCo2 cells [42] and that taxifolin inhibits triglyceride biosynthesis in HepG2 cells as a result of a noncompetitive type of DGAT1 inhibition [43]. Furthermore, the suppression of DGAT1 activity by taxifolin is due to a posttranscriptional mechanism of action [43].

We have recently demonstrated that macrophage PON2 protects the cells from triglyceride accumulation, secondary to its ability to inhibit DGAT1 activity and the triglyceride biosynthesis rate [26, 27]. As PJ and punicalagin upregulated PON2 expression in J774A.1 macrophages [28] as well as in MPM from C57BL/6 mice [29; present study], we questioned whether the inhibitory effect of PJ or punicalagin on macrophage triglyceride accumulation is mediated by PON2. To test this possibility we used MPM from PON2-deficient mice. However, in PON2-deficient MPM, the cellular triglyceride content, DGAT1 activity, and triglyceride biosynthesis rate were all inhibited by PJ and punicalagin, suggesting a direct effect of PJ and punicalagin on macrophage triglyceride accumulation. The effects of the major pomegranate polyphenol punicalagin were more pronounced than the effects of PJ. We suggest that PJ polyphenols, such as punicalagin, could possibly bind to the enzyme active site, leading to an inhibition of its activity.

After a single oral dose of PJ concentrate (180 ml containing 318 mg punicalagin and 12 mg of free ellagic acid), ellagic acid increased rapidly and was cleared from the plasma samples of all volunteers after 5 h. The maximum plasma concentration (Cmax) was 0.06 ± 0.01 μM [44].

Upon consumption of PJ, peritoneal macrophages are not exposed directly to PJ compounds or to punicalagin.

Fig. 4. Effect of PJ on triglyceride metabolism in PON2-deficient MPM: a concentration study. MPM were isolated from PON2-deficient mice at the age of 5 months. a, b The cells were incubated for 20 h at 37°C with increasing concentrations (0–50 μM) of PJ. The cellular triglyceride content (a) and the triglyceride biosynthesis rate (b) were determined as described in Methods. c Microsomes were isolated from PON2-deficient MPM. PJ (50 μM) was added directly to the microsomes, and DGAT1 activity was determined as described in Methods. Results are expressed as the mean ± SD of 3 different experiments. * p < 0.01 vs. concentration 0; # p < 0.01 vs. control cells.
(the bioavailability of which is very poor). However, we have previously demonstrated in several in vivo studies (both in mice and humans) [13, 14, 16, 17, 19, 20] the antiatherogenic effects of PJ consumption on serum and peritoneal macrophages. In parallel to the current in vitro study, we performed a study in which we analyzed the antiatherogenic effects of PJ consumption on peritoneal macrophages from C57BL/6 mice, PON1-deficient mice, and PON2-deficient mice [29]. In this study, we have clearly demonstrated that PJ consumption inhibited triacylglyceride biosynthesis by peritoneal macrophages derived from all 3 groups of mice, which is similar to the results observed in the current in vitro study. It is important to note that the antioxidant effects of PJ, or its metabolites, on the intestine are reflected on tissues, including blood serum and blood monocytes. Upon PJ consumption, its ellagitannins are hydrolyzed to release ellagic acid which is then converted by the gut microflora to urolithin derivatives. These urolithins may contribute to the biological effects of PJ as they may persist in serum and in some tissues (prostate, colon, and intestine) [44, 45]. Unlike in human studies, in rats that consumed a diet containing 6% punicalagin, punicalagin was detected in plasma at a concentration around 30 µg/ml [46].

Triglyceride accumulation in macrophages can lead to foam cell formation and atherosclerosis development since it increases mitochondrial reactive oxygen species production and results in cellular necrosis [9], inducing a unique pathway of lipotoxicity which is independent of the cellular degradation of triglycerides to free fatty acids [47]. We thus conclude that the ability of PJ polyphenols to protect against macrophage triglyceride accumulation is an important contributor to the antiatherogenic properties of pomegranate.

Disclosure Statement
All authors declare they have no financial/commercial conflicts of interest.
PJ Inhibits Macrophage Triglyceride Biosynthesis


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