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Postprandial triglyceride-rich lipoproteins regulate perilipin-2 and perilipin-3 lipid-droplet-associated proteins in macrophages☆

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Abstract

Lipid accumulation in macrophages contributes to atherosclerosis. Within macrophages, lipids are stored in lipid droplets (LDs); perilipin-2 and perilipin-3 are the main LD-associated proteins. Postprandial triglyceride (TG)-rich lipoproteins induce LD accumulation in macrophages. The role of postprandial lipoproteins in perilipin-2 and perilipin-3 regulation was studied.

TG-rich lipoproteins (TRLs) induced the levels of intracellular TGs, LDs and perilipin-2 protein expression in THP-1 macrophages and in Apoe$^{-/-}$ mice bone-marrow-derived macrophages with low and high basal levels of TGs. Perilipin-3 was only synthesized in mice macrophages with low basal levels of TGs. The regulation was dependent on the fatty acid composition of the lipoproteins; monounsaturated and polyunsaturated fatty acids (PUFAs) more strongly attenuated these effects compared with saturated fatty acids. In THP-1 macrophages, immunofluorescence microscopy and freeze-fracture immunogold labeling indicated that the lipoproteins translocated perilipin-3 from the cytoplasm to the LD surface; only the lipoproteins that were rich in PUFAs suppressed this effect. Chemical inhibition showed that lipoproteins induced perilipin-2 protein expression through the peroxisome proliferator-activated nuclear receptor (PPAR) PPARα and PPARγ pathways.

Overall, our data indicate that postprandial TRLs may be involved in atherosclerotic plaque formation through the regulation of perilipin-2 and perilipin-3 proteins in macrophages. Because the fatty acid composition of the lipoproteins is dependent on the type of fat consumed, the ingestion of olive oil, which is rich in monounsaturated fatty acids, and fish oil, which is rich in omega-3 fatty acids, can be considered a good nutritional strategy to reduce the risk of atherosclerosis by LD-associated proteins decrease.
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1. Introduction

Cytosolic lipid droplets (LDs) are physiologically important because certain human diseases such as atherosclerosis, steatosis, diabetes, obesity and cancer, all of which are major health problems of global interest, have been correlated with LD formation [1–3]. Structurally, LDs consist of a neutral lipid core surrounded by a phospholipid monolayer that contains a diverse array of embedded proteins [4]. The proteome of LDs includes structural proteins of the perilipin (PLIN) family (PLIN1–PLIN5), which play active roles in intracellular lipid storage, nascent LD biogenesis and the regulation of lipolysis of the lipids stored in these particles [5].

Excessive lipid accumulation by macrophages and their conversion to foam cells is a hallmark of atherosclerosis through all stages of lesion development. PLIN2 (ADRP, adipophilin) is the most prominent LD-associated protein in macrophages; it plays a key role in foam cell formation and in the pathogenesis of atherosclerosis [6] and has been localized in lipid-rich macrophages in atherosclerotic plaques of human carotid endarterectomized specimens and coronary arteries [7,8]. The macrophage expression of PLIN2 is up-regulated by agonists of the peroxisome proliferator-activated nuclear receptor (PPAR) γ (PPARγ) [9] through the AP-1 site in the PLIN2 promoter [10] and the ERK1/2 signaling pathway [11]. PLIN3 (TIP47) is fundamental to the formation and function of LDs and is involved in triglyceride (TG)

Abbreviations: BMDM, bone-marrow-derived macrophage; HFLCD, high-fat low-cholesterol diet; LD, lipid droplet; LFD, low-fat diet; MUFA, monounsaturated fatty acid; PPAR, peroxisome proliferator-activated nuclear receptor; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TG, triglyceride; TRL, TG-rich lipoprotein.

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metabolism [12]. The stimulation of PLIN3 expression leading to LD formation occurs primarily by promoting TGs but not cholesterol accumulation [13]. Nonesterified long-chain fatty acids act as natural ligands of PPARs and increase PLIN2 expression in a number of cells [14]; they also modify LD-associated proteins at the transcriptional level in macrophages [15] and effectively induce LDs in human hepatocytes [16]. They are also able to bind PLIN2 and PLIN3 [12]. In this manner, PLIN3 acts as a carrier protein for free fatty acids and participates in the conversion of macrophages to foam cells.

Postprandial hypertriglyceridemia is characterized by an increase in TG-rich lipoproteins (TRLs) in the circulating blood after the ingestion of an acute high-TG meal. TRLs are peculiar lipoproteins in the sense that they faithfully represent the fatty acid composition of the dietary fat ingested [17] and therefore enable an exceptional physiological approach to study the effects of dietary fatty acids on atherosclerosis. Human atherosclerotic plaques contain intact TRLs [18], which indicates that postprandial fat deposition contributes significantly to human atherogenesis. Macrophages take up TRLs through the apoB48 receptor, resulting in LD accumulation and foam cell formation [19]. In spite of its importance, the molecular basis for TRL-driven LD biogenesis and their pathogenicity is poorly understood.

The present study was designed to determine the role of TRLs of different fatty acid compositions on the regulation of PLIN2 and PLIN3 proteins in human THP-1-derived macrophages and in bone-marrow-derived macrophages (BMDMs) that were isolated from Apoe$^{-/-}$ mice fed a low-fat diet (LFD) or three high-fat low-cholesterol diets (HFLCDs) of different fatty acid compositions. We used refined olive oil, which is rich in monounsaturated fatty acids (MUFAs), or a mixture of refined olive and fish oils, which is rich in polyunsaturated fatty acids (PUFAs) due to their cardioprotective effect; we compared these with an atherogenic fat (butter) that is rich in saturated fatty acids (SFAs).

2. Materials and methods

2.1. Subjects, intervention and isolation of TRLs

Fourteen healthy Caucasian male nonsmokers [means±S.D.: body mass index, 23.9±1.9 kg/m^2; 22–37 years (range): 27±7 years] participated in this study. Briefly, this was a randomized crossover study. After 12 h of fasting, subjects consumed a meal supplemented with butter, refined olive oil or refined olive oil enriched with 3% fish oil. Meals consisted of a portion of plain pasta (30 g/m^2 body surface area), one slice of brown bread, one skimmed yogurt and either fat (50 g/m^2 body surface area). After the ingestion of the meals, blood samples were collected at the postprandial peak (maximum level of TGs) into serum separator tubes on gel Z for isolation of TRLs. Details of the study design have been reported previously [20]. All protocols were approved by the Human Clinical Commission and the Ethics Committee of Hospitales Universitarios Virgen del Rocio, Seville. The study was carried out in accordance with the principles outlined in the Helsinki Declaration.

Human TRLs were isolated by ultracentrifugation and identified as described [21]. Lipoproteins were pooled, dialyzed against phosphate-buffered saline (PBS) and immediately stored at −80°C. Only once-thawed, postprandial TRLs samples were used for cell culture studies. Fatty acid composition (Table 1), TG concentration, oxidation levels and possible endotoxin contamination in the TRLs were determined as described [17].

2.2. Animal diets and experimental design

Male Apoe$^{-/-}$ mice bread onto a C57BL/6J background (The Jackson Laboratory, Bar Harbor, ME) were used for the study. HFLCDs were provided in pellets that contained 19.5% as proteins, 20% as fat (0.01% as cholesterol) and 50% as carbohydrates. The different fats provided in the pellets were the same as those used for the human studies: butter (HFLCD-SFAs), olive oil (HFLCD-MUFAs) or refined olive oil plus 3% fish oil (HFLCD-PUFAs) (Supplemental Table 1). The control LFD (Teklan global 14% protein rodent maintenance diet; Harlan Laboratories Inc., Barcelona, Spain) contained 14.3% crude protein, 4.0% fat (0% cholesterol), 48.0% carbohydrates and 22.5% fiber. After weaning, the mice were randomly allocated into 4 groups (n=12 per group) as follows: (1) group received the LFD, (2) the HFLCD-SFAs, (3) the HFLCD-MUFAs or (4) the HFLCD-PUFAs for 12 weeks. At 18 weeks of age, the animals were fasted overnight and euthanized with an overdose of pentobarbital (1:10 in PBS, 150 mg/kg, ~200 μl). Cells from the bone marrow were collected and differentiated into macrophages. Experiments were performed at the animal facility of the Faculty of Medicine, University of Seville. All animal protocols received appropriate institutional approval (Animal Care and Use Committee of the University of Seville) and were performed according to the official rules formulated in the Spanish law on the care and use of experimental animals.

Table 1
Fatty acid composition of TRLs isolated from blood drowned 3 h after the consumption of a meal enriched in SFAs, MUFAs or PUFAs.

	TRLs-SFAs	TRLs-MUFAs	TRLs-PUFAs
	(% by weight of total fatty acids)		
Total-SFAs	54.6±1.7^a	18.4±2.1^b	17.7±0.4^b
Palmitic acid (16:0)	35.6±1.1^a	13.1±0.7^b	11.0±0.5^c
Total-MUFAs	34.7±1.6^c	73.5±3.4^a	65.8±1.2^b
Oleic acid (18:1n-9)	32.0±0.4^c	72.1±2.9^a	67.2±0.5^b
Total-PUFAs	10.6±0.5^b	8.5±0.6^c	16.5±0.9^a
Eicosapentaenoic acid (20:5n-3)	0.1±0.0^b	0.1±0.0^b	5.9±0.2^a
Docosahexaenoic acid (22:6n-3)	0.2±0.0^b	0.3±0.2^b	1.8±0.1^a

Values are means±S.D., n=3.
Different superscript letters (a–c) denote that mean values in a row with different letters are significantly different (P<.05).

2.3. Isolation of bone marrow cells and differentiation into BMDMs

Cells from bone marrow were isolated and pooled for each group of mice. Femora and tibiae were aseptically removed and dissected free of adherent soft tissue. The bone ends were cut, and the marrow cavity was flushed out into a petri dish by slowly injecting ice-cold PBS solution at one end of the bone using a sterile 21-gauge needle. The bone marrow suspension was carefully agitated with a plastic Pasteur pipette to obtain a single cell suspension. The cells were washed and depleted of red blood cells using a hypotonic lysis buffer. After washing twice with PBS, the cells were cultured and differentiated into macrophages for 7 days at 37°C under a humidified atmosphere containing 5% CO_2 in 12-well dishes containing supplemented RPMI 1640 medium (50 units/ml penicillin G, 50 μg/ml streptomycin, 2 mM glutamine) plus 10% heat-inactivated fetal bovine serum (FBS) and 15% (v/v) L929-cell conditioned medium.

2.4. Cell culture conditions and TRL stimulation of human and mice macrophages

Human monocytic THP-1 cells (American Type Culture Collection TIB-22, Barcelona, Spain) were cultured in supplemented RPMI 1640 medium with 10% FBS. THP-1-derived macrophages were obtained after incubating THP-1 monocytes with PMA (100 nM; Sigma-Aldrich, St. Louis, MO) for 72 h. The THP-1-derived macrophages were then exposed to supplemented RPMI medium containing 50 μg/ml TGs of TRLs-SFAs, TRLs-MUFAs or TRLs-PUFAs for 48 h. For the inhibition studies, THP-1-derived macrophages were preincubated with 4 μM GW9662 (PPARγ antagonist; Sigma), 4 μM MK886 (PPARα antagonist; Sigma), 2 μM GSK0660 (PPARδ/β antagonist; Sigma) or a mix of them for 1 h followed by 50 μg/ml TGs of TRLs-SFAs for 48 h. For the mice cells, the BMDMs were cultured in supplemented RPMI 1640 medium, adding 15% L929-cell conditioned medium and 10% FBS. To induce lipid-laden BMDMs, the cells obtained from the mice fed the LFD (BMDMs-LFD) were incubated with 50 μg/ml TGs of TRLs-SFAs, TRLs-MUFAs or TRLs-PUFAs for 48 h. Additionally, to study the effect of high basal TGs content in macrophages on the induction of LD-associated proteins, the cells obtained after feeding the mice with the HFLCD-SFAs (BMDMs-HFLCD-SFAs) were stimulated with only 50 μg/ml TGs of TRLs-SFAs; BMDMs-HTGD-MUFAs were incubated with TRLs-MUFAs and BMDMs-HTGD-PUFAs were stimulated with TRLs-PUFAs. A total of four pooled samples from BMDMs-LFD and BMDMs-HFLCDs prior to and after TRL stimulation (lipid-laden BMDMs) were evaluated in triplicate for TGs, PLIN2 and PLIN3 protein expression (Supplemental Fig. 1).

2.5. Measurement of the lipid content in lipid-laden macrophages

The cells were washed twice with PBS and scraped in 400 μl of 0.9% NaCl. After sonication, the protein content of the lysate was measured using the Bradford protein assay (Bio-Rad Laboratories, Madrid, Spain). Cellular lipids were extracted using hexane/isopropanol (3:2, v/v). The TG and cholesterol contents were measured using the assay kits GPO/PAP (Axiom Diagnostics, Bürstadt and Worms, Germany) and CHOD/PAP (ProDiagnostica, Germany), respectively. For the identification of the neutral lipids in LDs, the cells were fixed with 4% formaldehyde for 30 min. The cell lipids were stained with oil red O (2 mg/ml in 60% isopropanol; Sigma-Aldrich) for 5 min. Images were captured using an Olympus IX81 inverted phase microscope (Olympus Corporation, Barcelona, Spain).

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