Triacylglycerol-Rich Lipoproteins Interact with Human Vascular Cells in a Lipid-Dependent Fashion

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Plasma triacylglycerol-rich lipoproteins (TRL) are being considered as a key lipid fraction in the pathogenesis of atherosclerotic cardiovascular disease. Here we compared the influence of two monounsaturated oils [virgin olive oil (VOO) and high-oleic sunflower oil (HOSO)] on the capability of postprandial TRL to interact with two human vascular cell lines [umbilical vein endothelial (HUVEC) and aorta smooth muscle (HASMC) cells]. A fluorescent probe was used for labeling TRL and to determine receptor activity of HUVEC and HASMC. The values for total cell-associated, bound, and internalized TRL were higher in HUVEC, and TRL from VOO was the better ligand recognized but at lower affinity than TRL from HOSO. There was a competitive effect of very low density lipoproteins (VLDL) for the uptake of TRL by cells, which was found to be dependent on the origin/lipid composition of the ligands and cell-type specific. We also conclude that the VLDL receptor (VLDLr) may contribute significantly to the HASMC binding capacity for postprandial TRL mediated by lipoprotein lipase (LPL) or LPL-binding molecules. Our findings are compatible with a selective role of the clustered O-linked sugar domain of the VLDLr in the catabolism of TRL by human vascular cells.

Keywords: *Triacylglycerol-rich lipoproteins; human vascular cells; membrane receptors; dietary monounsaturated oils*

INTRODUCTION

Elevated levels of cholesterol-rich lipoproteins in plasma have been shown to contribute to endothelial dysfunction. This damage is present not only in the early stages of atherosclerosis but also persists in later events (*1*, *2*). There is an increase in the permeability of the endothelium to lipoproteins, monocytes, and macrophages, which may increase smooth muscle cell migration and proliferation (*3*), aiding in the formation of an intermediate lesion and progression to an atherosclerotic plaque (*4*). However, little is known about the interaction of triacylglycerol-rich lipoproteins (TRL) with vascular cells. While there is emerging evidence that relate premature coronary heart disease and the metabolism of TRL (*5*, *6*), there is particular emphasis on the rate of clearance of postprandial TRL that is actually been considered as an independent cardiovascular risk factor (*7*, *8*).

The major uptake of fasting TRL (very low density lipoproteins, VLDL) into cells has been proposed to be mediated by the VLDL receptor (VLDLr) (*9*). The mRNA for the VLDLr is abundant in heart, skeletal muscle, adipose tissue, kidney, and brain but is not detectable in the liver. Interestingly, VLDLr is also expressed in the vascular endothelium of large vessels and capillaries (*10*) and in vascular smooth muscle cells within atherosclerotic lesions from humans (*11*). The distribution pattern of VLDLr coincides with that of lipoprotein lipase (LPL), but differs from the distribution of the other members of the low-density lipoprotein (LDL) receptor (LDLr) supergene family (*12*). This suggests that LPL has a dual role as an hydrolytic enzyme of core triacylglycerols in circulating TRL and as a bridging protein that increases the binding of TRL to cell surfaces (*13*).

In endothelial cells, the VLDLr binds to and mediates the catabolism of LPL (*14*). The enzyme can also be associated to heparan sulfate proteoglycans (HSP), metabolized at a slow rate, and recycled back to the cell surface, without requiring transfer to VLDLr (*15*). In smooth muscle cells, VLDL−LPL complex can be cell-associated by two pathways, one involving VLDLr that undergoes rapid endocytosis in clathrin-coated pits and a second probably involving direct uptake by HSP (*16*). These findings indicate that TRL can be differentially catabolized in vascular cells, which is of major importance for the residence time of lipoproteins in plasma and the utilization of the lipids that they transport.

The diet is one of the major determinants in the postprandial levels of triacylglycerols. Different studies have stressed the influence of the amount and type of food in coronary vascular events (*17*−*19*). Polyunsaturated (PUFA) and monounsaturated (MUFA) fatty acids are associated to a lower postprandial lipaemia and to a higher TRL clearance when compared with saturated fatty acids (SFA) (*20*, *21*). These findings reinforce the

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Table 1. Clinical and Biochemical Characteristics of the Participants[a]

age (years)	27 ± 7
body mass index (kg/m^2)	22.7 ± 1.7
total cholesterol (mmol/L)	4.1 ± 0.5
HDL cholesterol (mmol/L)	1.6 ± 0.3
LDL cholesterol (mmol/L)	2.3 ± 0.4
triacylglycerols (mmol/L)	0.8 ± 0.2
phospholipids (mmol/L)	58.3 ± 5.0
apolipoprotein A-I (g/L)	1.1 ± 0.1
apolipoprotein B (g/L)	0.7 ± 0.1

[a] Values are mean ± SD (n = 8).

idea of a link between food composition and primary development of cardiovascular disease.

In an attempt to gain insight into the metabolism of TRL in humans and its relationship with food composition, we demonstrated that the ingestion of two highly monounsaturated oils [virgin olive oil (VOO) and high-oleic sunflower oil (HOSO)] showed different triacylglycerol postprandial response, suggesting a role of the triacylglycerol composition of TRL (*22*). Accordingly, a recent review (*23*) pointed out that the atherogenicity of postprandial lipoproteins could be a non-apolipoprotein B-mediated process but a lipid-mediated process.

In the present study, we used postprandial TRL labeled with a fluorescent probe (DiI) to determine their interaction with human vascular cells, such as umbilical vein endothelial (HUVEC) and aorta smooth muscle (HASMC) cell lines. TRL were obtained after the ingestion of a meal rich in VOO or in HOSO. We considered the influence of the composition of TRL triacylglycerols on TRL uptake by HUVEC and HASMC to be particularly important because postprandial TRL are selectively cleared in humans (*22*, *24*) and trigger cellular activation of the mitogen-activated protein kinase pathway (unpublished data) depending on the lipid composition of the lipoproteins. It is interesting to note that VOO and HOSO have similar effects on cholesterol metabolism (*25*, *26*), but they differ in the effects on triacylglycerol metabolism (*27*, *28*). We also determined the expression of VLDLr in HASMC, which may be subjected to posttranscriptional differential splicing of the clustered O-linked sugar domain.

MATERIALS AND METHODS

Subjects and Experimental Design. Eight healthy male volunteers were enrolled for the study. All subjects were normolipidemic, had a normal body mass index, and none had a history of cardiovascular disease and did not suffer from any digestive disease (Table 1). Following a 12-h fasting period, subjects ingested, on separate occasions, three meals with 2 wk between meals. In a randomized order, the meals consisted of one slice of brown bread (28 g), plain pasta (100 g), tomato sauce (130 g), and one skimmed yogurt (meal 1: basal meal), supplemented with virgin olive oil (VOO) (70 g) (meal 2: meal enriched in VOO) or high-oleic sunflower oil (HOSO) (70 g) (meal 3: meal enriched in HOSO). The fatty acid and triacylglycerol molecular species composition of the oils is depicted in Table 2. Meals 1, 2, and 3 provided 1936, 4523, and 4523 kJ, respectively. The composition of meals 2 and 3 was as follows: 60% lipids (45% oleic acid), 33% carbohydrates, and 8% protein. No additional food was allowed throughout the postprandial period after each meal, and they drank only water. The experience was conducted as a short-term double-blinded study, where the participants were their own control in a crossover design. The protocol was reviewed and approved by the Institutional Committee on Investigation in Humans (Hospitales Universitarios Virgen del Rocío, Seville), and the experimentation was carried out in accordance with the Declaration of Helsinki of the World Medical Association. Informed consent was received from all participants.

Table 2. Fatty Acid and Triacylglycerol Molecular Species Composition of the Oils[a]

	VOO	HOSO
fatty acid		
16:0	12.8 ± 0.7[a]	4.1 ± 0.1[b]
16:1n-7	1.1 ± 0.1[a]	0.1 ± 0.0[b]
18:0	2.7 ± 0.5[b]	4.0 ± 0.1[a]
18:1n-9	75.9 ± 2.2[b]	77.3 ± 1.1[a]
18:2n-6	5.6 ± 0.8[b]	14.1 ± 0.1[a]
18:3n-3	0.9 ± 0.1[a]	0.1 ± 0.0
others	1.0	0.3
triacylglycerol		
LOO	4.1 ± 0.6[a]	2.6 ± 0.4[b]
POL/LLS	1.2 ± 0.2[a]	0.4 ± 0.1[b]
OOO	62.0 ± 4.4[b]	86.7 ± 2.6[a]
POO	29.4 ± 3.2[a]	3.5 ± 0.9[b]
SOO	1.8 ± 0.2[b]	3.6 ± 0.7[a]
others	1.5	3.2

[a] Values are mean ± SD (n = 3). Composition is in g/100 g of total fatty acids or triacylglycerols. Data in a row with different letters are significantly different, $P < 0.05$. Abbreviations: VOO, virgin olive oil; HOSO, high-oleic sunflower oil; L, linoleic acid (18:2n-6); O, oleic acid (18:1n-9); P, palmitic acid (16:0); S, stearic acid (18:0); LOO, linoleoyl-dioleoyl-glycerol; POL, palmitoyl-oleoyl-linoleoyl-glycerol; LLS, dilinoleoyl-stearoyl-glycerol; OOO, triolein; POO, palmitoyl-dioleoyl-glycerol; SOO, stearoyl-dioleoyl-glycerol.

Lipoprotein Isolation and Labeling. According to previous studies (*22*, *24*), the kinetics of postprandial TRL (Sf > 400, density > 0.93 g/mL) for 7 h following VOO- or HOSO-enriched meals were characterized by a biphasic triacylglycerol response. The maximum level was found at 2 h, and a small peak at 4 h for VOO and at 5 h for HOSO was also detected. The lipid composition of TRL at 2 h was similar either for TRL from VOO [cholesteryl esters (CE), 5.4%; phospholipids (PL), 13.6%; and triacylglycerols (TG), 81%] or from HOSO (CE, 6.6%; PL, 15.0%; TG, 78.4%). For the present study, TRL were isolated from the plasma of the participants before the ingestion of the meal and at 2 h afterward. A cubital vein was catheterized with a small bore extension set with SMARTSITE needle-less valve port equipped with a disposable vacutainer (Meylen, Cedex, France). Lipoprotein deficient serum (LPDS) was obtained from fasting serum (preincubated at 57 °C for 60 min) after removal of all lipoproteins at density 1.21 g/mL. For TRL isolation, postprandial plasma (4 mL) was layered with 6 mL of NaCl solution (density 1.006 g/mL) and centrifuged at 95000*g* for 42 min at 15 °C. LPDS and TRL preparations were extensively dialyzed against phosphate-buffered saline (PBS) containing EDTA 0.01% for 18 h at 4 °C and filtered through 0.22 μm (Nalgene, Rochester, NY) and 1.2 μm (Sigma, St. Louis, MO) size pore, respectively. Triacylglycerol (TG) concentration was measured in TRL (Boehringer-Mannheim, Meylen, France) and adjusted to 1000 μg/mL.

Apolipoproteins B-100 and B-48 were identified in the isolated TRL fraction as described (*29*). To prevent scission of apolipoproteins, benzamidine (0.3 g/L) was added to plasma preparations. Albumin was removed by centrifugation at 93000*g* for 18 h at 12 °C. Apolipoproteins were then resolved by SDS−PAGE (7.5% SDS−PAGE slab gels, 1.5 mm thick) according to their gel migration in relation to the migration of the Perfect Protein markers (MW 10−225 kDa) (Calbiochem-Novabiochem, Schwalbach, Germany) and of LDL as a B-100 standard (549 kDa). LDL and VLDL were isolated by cumulative rate centrifugation in a density gradient (*30*).

TRL were labeled with the fluorescent probe 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-indocarbocyanide perchlorate (DiI) (Molecular Probes, Leiden, The Netherlands) as described previously for LDL labeling (*31*) with minor modifications. A stock solution of DiI was prepared by dissolving 3 mg of DiI in 1 mL of DMSO. One mL of TRL (1000 μg TG/mL) was added to 2 mL of LPDS and mixed with 10 or 100 μL of DiI in DMSO (30 or 300 μg of DiI, respectively) to determine the labeling

efficiency of TRL. The mixture was incubated for 18 h at 37 °C in the dark. The DiI-labeled TRL were reisolated by ultracentrifugation, dialyzed against PBS-EDTA, and stored at 4 °C.

Standard solutions of DiI and of DiI-TRL were prepared in either chloroform or 2-propanol with a wide concentration range (0 to 100 ng of DiI/mL and 0 to 100 μg of TG/mL). Fluorescence was determined in a SLM Aminco Bowman series 2 Luminescence spectrometer (Urbana, IL) and in a Tecan SpectraFluor plate reader (Grödig/Salzburg, Austria) with 520 nm excitation and 580 nm emission wavelengths. The specific activity of DiI-TRL was then estimated as the amount of DiI (ng) incorporated into 1 μg of TRL TG.

Cell Culture. The human umbilical vein endothelial cell line CRL-1730 (HUVEC) and the human aorta smooth muscle cell line CRL-1999 (HASMC) were obtained from the ATCC (Rockville, MD). HUVEC were allowed to grow in Medium 199 (BioWhittaker, Walkersville, MD) supplemented with FCS (20% v/v), penicillin−streptomycin (50 U mL^{-1}), hepes (20 mM), endothelial cell growth supplement (30 μg mL^{-1}), and heparin (100 μg mL^{-1}). HASMC were grown in Medium 199 (GibcoBRL, Life Technologies, Barcelona, Spain) supplemented with FCS (10% v/v), penicillin−streptomycin (50 U mL^{-1}), and L-glutamine (2 mM). Cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2. Cells were used after 5 to 15 passages for HUVEC and after 3 to 10 passages for HASMC. When confluence was reached, the cells were detached by trypsinization (trypsin 0.05% plus EDTA 0.02%) and subsequently subcultured in flasks under the same conditions described above or seeded in 96 MicroWell flat bottom plates (Nunc, Roskilde, Denmark).

Bovine Milk Lipoprotein Lipase Purification. LPL was purified from fresh bovine milk as previously described (*32*). The enzyme preparation was first stored at −70 °C in the presence of 30% glycerol, thawed, and extensively dialyzed against PBS at 4 °C before LPL was used for experiments. The purity of the enzyme (>95%) was established by SDS−PAGE (12.5% SDS−PAGE slab gels, 1.5 mm thick). Protein was determined by the method of Bradford (Bio-Rad, München, Germany).

Cell-Interaction of triacylglycerol-Rich Lipoproteins. After 96 h in culture with complete medium, subconfluence HUVEC seeded in 96 MicroWell plates were made quiescent by incubation in Medium 199 supplemented only with penicillin−streptomycin (50 U mL^{-1}), and hepes (20 mM) for another 24 h. HASMC were also synchronized by incubation in serum-free medium for 48 h. To establish the dose−response of bound and cell-associated TRL, DiI-TRL (0−150 μg of TG/mL) from either virgin olive oil or high-oleic sunflower oil were incubated with vascular cells for 2 h at 4 and 37 °C, respectively. All incubations were performed in quadruplicate. Cells were carefully washed three times with cold PBS and 200 μL of 2-propanol was added to each well. The fluorescence of the 2-propanol extract of DiI was directly measured (a single step) in a Tecan SpectraFluor plate reader. All fluorometric data were corrected for the autofluorescence of the cells incubated with medium alone. The dose−response of internalized TRL was calculated as the difference between cell-associated (37 °C) and membrane-bound (4 °C) TRL. Cellular protein was dissolved in NaOH 0.1 N and determined by the method of Bradford. The results were expressed as micrograms of TG of DiI-TRL per milligram of cell protein. Scatchard plot analysis was used for the calculation of K_m and maximal capacity of DiI-TRL uptake (*33*).

The specificity of cell-associated TRL was determined by incubation of DiI-TRL with a 5-fold excess of unlabeled TRL and VLDL. Kinetics of cell-associated TRL were carried out by incubation of cells with DiI-TRL (50 μg of TG/mL) at different times (0 to 120 min). For binding of LPL to HUVEC and HASMC, quiescent cells were preincubated for 2 h at 4 °C with purified bovine milk LPL (1−8 μg/mL) and carefully washed two times with cold serum-free medium to remove any unbound LPL. The role of LPL in the recognition and uptake of TRL by vascular cells was also studied by using the monoclonal antibody (MAb) 5D2 (a generous gift of Dr. John D. Brunzell, Department of Medicine, University of Washington, USA). For that purpose, HUVEC and HASMC were preincubated for 1 h at 37 °C with MAb 5D2 (5 μg/mL) and carefully washed two times with serum-free medium before the addition of TRL.

VLDL Receptor Expression in HASMC. RNA isolation from HASMC was performed according to standard procedures. For PCR amplification of human VLDLr mRNA, 1 μg of total RNA was reverse-transcribed using random primer hexamers. The following oligonucleotides were used for PCR primers: WBV-1, 5′-ATGGAGGATGTGAATACC-3′; WBV-17, 5′-GCTCTGGTCACATTGATC-3′. PCR amplifications of cDNA were performed with the two pairs of primers, WBV-1/WBV-17 (4 min at 97 °C, 35 cycles of 30 s at 94 °C, 30 s at 50 °C, and 30 s at 72 °C) and the GeneAmp RNA PCR and PCR kit (Perkin-Elmer, Branchburg, NJ) on a Perkin-Elmer thermal cycler model 2400. GAPDH specific primers (GAPDH-5′, 5′-TGATGACATCAAGAAGCTGGTGAAG-3′; GAPDH-3′, 5′-TCTTGGAGGCCATGTGGGCCAT-3′) were used as internal controls. PCR parameters for GAPDH-5′/GAPDH-3′ primers were 7 min at 94 °C, 35 cycles of 1 min at 94 °C, 1 min at 50 °C, and 1 min at 72 °C. DNA variant fragments were separated by SDS−PAGE (7.5% SDS−PAGE slab gels, 1.5 mm thick), and the relative amounts of the two different receptor variants were assessed by using the Fuji Bio-Image analyzer system Bas 2000 (Fuji Photo Film).

Lipid Analysis of Triacylglycerol-Rich Lipoproteins. Total lipids of TRL were extracted with chloroform/methanol (1:1, v/v) and dissolved in *n*-hexane. TG were isolated by solid-phase extraction (SPE) diol columns (Supelco, Bellefonte, PA) using hexane/methylene chloride (9:1, v/v) as eluent. An aliquot was taken for analysis of total fatty acids by GC using a model 5890 series II gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a flame-ionization detector and a capillary silica column Supelcowax 10 (Supelco) of 60 m length and 0.25 mm internal diameter (*28*). A second aliquot was stored at −20 °C for further analysis of the TG molecular species by HPLC using a model 2690 Alliance liquid chromatograph (Waters, Milford, MA) coupled to a light-scattering detector model DDL31 (Eurosep, Cergy-Pontoise, France) and a Spherisorb ODS-2 column (250 × 4.6 mm) of 3 μm particle size (Waters) (*22*). We considered all of the stereospecific positions in the glycerol molecule to be equivalent because HPLC cannot separate positional isomers (*27*, *34*). The system was controlled by computer through Millenium System (Waters).

Statistical Analysis. Data are expressed as means ± SD and were analyzed by analysis of variance for repeated measures and by using the Student's t-test for unpaired analysis. Differences were considered significant at $P < 0.05$.

RESULTS

Labeling of TRL with DiI. Lipoprotein labeling with the lipid-soluble fluorescent probe DiI was used to study the interaction of TRL with human vascular cells. First, we constructed a standard curve of DiI dissolved in 2-propanol or chloroform obtaining similar linear correlations ($R^2 = 0.9922$ or 0.9957) up to a concentration of 100 ng/mL (data not shown). As a single-step fluorometric assay, dilution of DiI-TRL in 2-propanol showed an excellent linear response of fluorescence intensity with minimum detection limit lower than 5 ng of DiI/mL (Figure 1A). In addition, the labeling efficiency of DiI-TRL was 9.7 and 28.2 ng of DiI/μg of TG for TRL from VOO at a ratio of 1000 μg of TG to 30 and 300 μg of DiI, respectively. While 12.6 and 60.2 ng of DiI/μg of TG was found for TRL from HOSO at a ratio of 1000 μg of TG to 30 and 300 μg of DiI (Figure 1B). DiI-TRL were prepared on four different occasions for uptake studies, and the specific activities obtained varied by less than 10%.

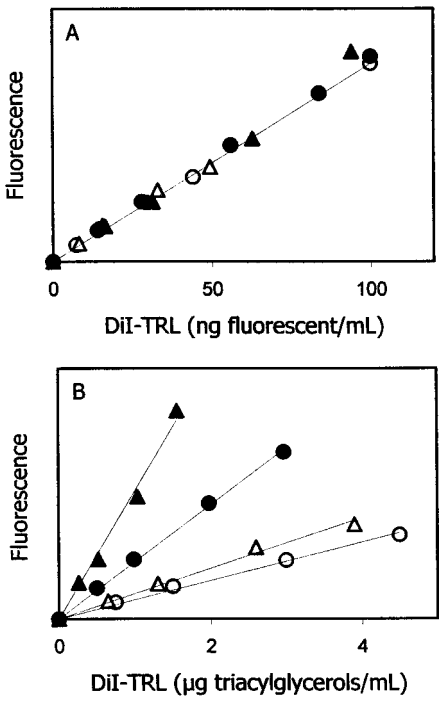

Figure 1. Fluorescence of labeled-TRL as a function of DiI (A) and triacylglycerol (B) concentration. The fluorescence intensities were linear ($R^2 > 0.99$) over the indicated range for DiI-TRL from VOO prepared at a ratio 30:1000 (open circles) and 300:1000 (filled circles), and for DiI-TRL from HOSO at a ratio 30:1000 (open triangles) and 300:1000 (filled triangles), (μg/μg).

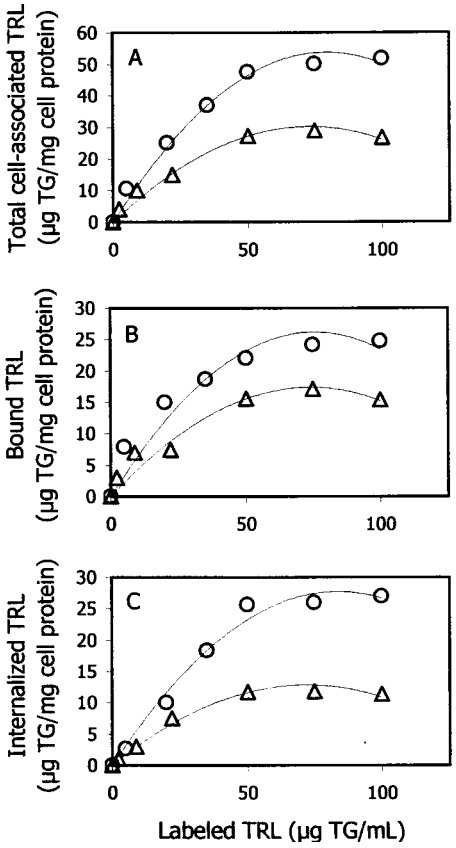

Figure 2. Dose-response measurement of cell-associated (A), bound (B), and internalized (C) DiI-TRL in HUVEC. Cells were cultured in 96 MicroWell flat bottom plates and incubated with 0−100 μg of DiI-TRL triacylglycerol/mL for 2 h at 37 °C (A) or 4 °C (B). Internalized TRL was calculated as the difference between A and B. After washing, the cells were dissolved in 200 μL of 2-propanol. TRL from VOO (open circles) or HOSO (open triangles) and labeled at a ratio 300:1000 (μg of DiI/μg of triacylglycerol) were used. Each point represents the mean of eight wells which varied by less than 10%.

Uptake of DiI-TRL by Vascular Cells. Cell-association, binding, and internalization of DiI-TRL by HUVEC and HASMC showed a concentration-dependent response (Figures 2 and 3). There was a linearity at a concentration range of 5 to 40 μg of TG/mL and appeared to reach saturation at 55 μg of TG/mL. Scatchard plot analysis displayed the apparent K_m and maximal capacity of DiI-TRL to be uptaken by the human vascular cells (Figure 4). TRL from VOO exhibited a higher apparent K_m and maximal capacities than TRL from HOSO (Table 3). Among cells, HUVEC presented lower values for apparent K_m and higher values for maximal capacity of DiI-TRL uptake than HASMC.

The incorporation of DiI-TRL had a higher significant nonspecific component for HUVEC (Figure 5), but unlabeled TRL and VLDL samples did compete for labeled TRL uptake. In addition, HUVEC and HASMC exhibited similar time-response for the uptake of the ligands either from VOO or HOSO, at the concentration of 50 μg of TG/mL (Figure 6). The saturation levels were reached at 120 min of incubation.

The role of LPL in the recognition and uptake of TRL by HUVEC and HASMC was studied by using purified LPL and a monoclonal antibody (5D2) against C-terminal LPL sequences to antagonize LPL function. First, the exofacial leaflet of the plasma membrane of vascular cells was labeled with increasing concentrations of LPL for 2 h at 4 °C. At enzyme concentrations greater than 5 μg/mL, the binding of LPL reached a plateau (data not shown). LPL had no effect on total cell-associated DiI-TRL in HUVEC (Figure 7). By contrast, there was a significant increase for the ligands cell association in HASMC with bound LPL. Similar increase was detected for TRL either from VOO or HOSO. MAb 5D2 and heparin partially inhibited the lipoprotein−cell interaction.

Expression of VLDL Receptor in HASMC. Previously, it was demonstrated that endothelial cells exclusively express a VLDLr isoform that lacks the O-linked sugar domain (*35*), which is involved in the stability of the receptor on the cell surface (*36*). To determine which RNA variant of the VLDLr is predominant in HASMC, the primers WBV-1 and WBV-17 (surrounding the O-linked sugar domain) were used for RT-PCR (Figure 8). Two bands of 220 and 136 bp were amplified that corresponded to the glycosylated and nonglycosylated region sequences, respectively. Semiquantitative analysis indicated that the O-linked sugar variant of the VLDLr was predominantly expressed by HASMC.

Lipid Composition of TRL. Differences were found in the fatty acid composition of the TG fraction of postprandial TRL from oils. The percentages of significant fatty acids were 14.1 (palmitic acid, 16:0), 66.0 (oleic acid, 18:1n-9), 7.8 (linoleic acid, 18:2n-6), and 0.8 (linolenic acid, 18:3n-3) for TRL from VOO, while 6.6, 70.7, 11.6, and 0.2 for TRL from HOSO. With regard to the composition of the TG molecular species, LOO (linoleoyl-dioleoyl-glycerol; 11.4%), POO (palmitoyl-dioleoyl-glycerol; 31.1%), and OOO (triolein; 43.6%) were the major TG found in TRL from VOO. By contrast, HOSO produced TRL with a higher content in OOO

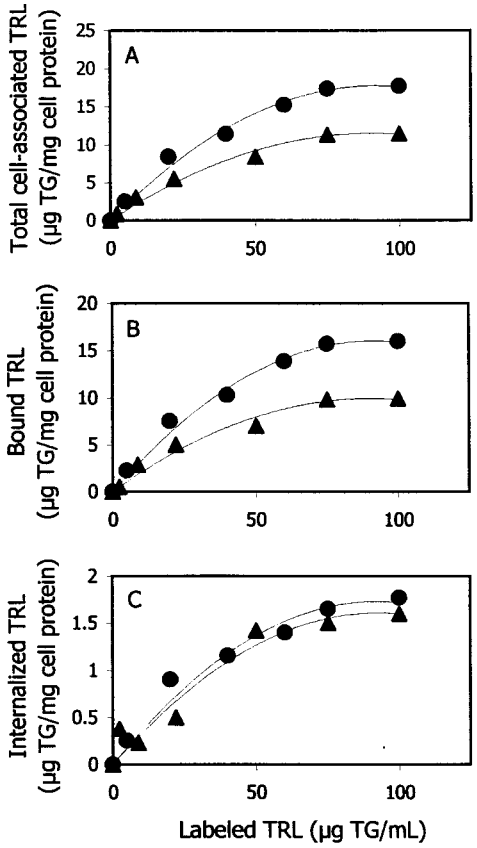

Figure 3. Dose-response measurement of cell-associated (A), bound (B), and internalized (C) DiI-TRL in HASMC. Cells were cultured in 96 MicroWell flat bottom plates and incubated with 0−100 μg of DiI-TRL triacylglycerol/mL for 2 h at 37 °C (A) or 4 °C (B). Internalized TRL was calculated as the difference between A and B. After washing, the cells were dissolved in 200 μL of 2-propanol. TRL from VOO (filled circles) or HOSO (filled triangles) and labeled at a ratio 300:1000 (μg of DiI/μg of triacylglycerol) were used. Each point represents the mean of eight wells which varied by less than 10%.

(60.5%) and LOO (25.0%) but with a lower content in POO (7.4%).

DISCUSSION

This study shows that postprandial TRL can differentially be uptaken by two human vascular cell lines. This uptake was also dependent on the lipid composition of the lipoprotein particles.

The fluorochrome DiI was used as a nonradioactive approach for labeling TRL. Its potential application for the binding and intracellular trafficking of lipoproteins (LDL and VLDL) by adherent and nonadherent cultured cells has been yet demonstrated (*31*, *33*, *37*). As shown by the linear solubilization capacity that overexceeded the mass of DiI to be associated with vascular cells, 2-propanol was selected to quantitatively extract DiI for fluorescence determination in a single step. This plastic-compatible organic solvent directly allowed a fast detection of fluorescence intensity of the DiI-label in microplates for cell culture with high sensitivity and accuracy. The labeling efficiency of DiI-TRL was defined by two ratios of TG (TRL) and DiI, 1000:300 and 1000:30 (μg/μg). It appeared that TRL from HOSO were more efficiently labeled with DiI than TRL from VOO, and the incorporation of DiI into TRL did not proportionally increase with the concentration of DiI. These differences in the efficiency of DiI labeling can be ascribed to the

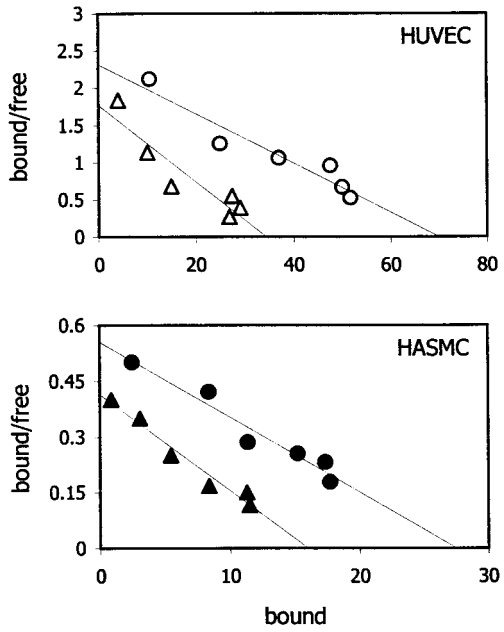

Figure 4. Scatchard analysis of the DiI-TRL uptake by vascular cells. Scatchard plot analysis of the data from Figures 2 and 3 when cells were incubated for 2 h at 37 °C. The lines represent the calculated best fit of the data. TRL from VOO (circles); TRL from HOSO (triangles).

Table 3. Kinetic Parameters of Scatchard Analysis for the Uptake of DiI-TRL by Human Vascular Cells[a]

		K_m (μg of TG/mL)	maximal capacity (μg of TG/mg of cell protein/2 h)
HUVEC			
	VOO	30	70
	HOSO	19	34
HASMC			
	VOO	50	28
	HOSO	39	16

[a] Each data represents the mean of eight wells which varied by less than 10%. Abbreviations: TRL, triacylglycerol-rich lipoproteins; HUVEC, human umbilical vein endothelial cells; HASMC, human aorta smooth muscle cells; VOO, virgin olive oil; HOSO, high-oleic sunflower oil; TG, triacylglycerols. VOO and HOSO mean TRL after the ingestion of VOO and HOSO, respectively.

spatial distribution of the fluorescent probe in either the surface and/or the core of the lipoproteins. The nature of DiI, a long-chain dialkylcarbocyanine, makes it a highly lipophilic tracer that would be better incorporated into an apolar environment. The composition of TG of TRL shows that TRL from VOO had a more apolar core than TRL from HOSO, which is contrary to the polarity found in the phospholipid surface (unpublished data). Therefore, our findings agree with the association of DiI with the surface of TRL (*38*).

There is virtually no information on the receptor activity of vascular cells to postprandial TRL. The current model states that the binding and uptake of lipoproteins are mediated by LPL acting as a bridge between the lipoproteins and proteoglycans that are present on the plasma membrane. The dose−response studies on cell-association, binding, and internalization indicated that HUVEC interact at higher affinity than HASMC with TRL, and that the ligand from VOO is better recognized by both cell lines. These results suggest as expected that the cell surface molecular organization of HUVEC is adapted to the clearance of plasma lipoproteins. In fact, the internalization of TRL in HASMC was low and the cells did not distinguish

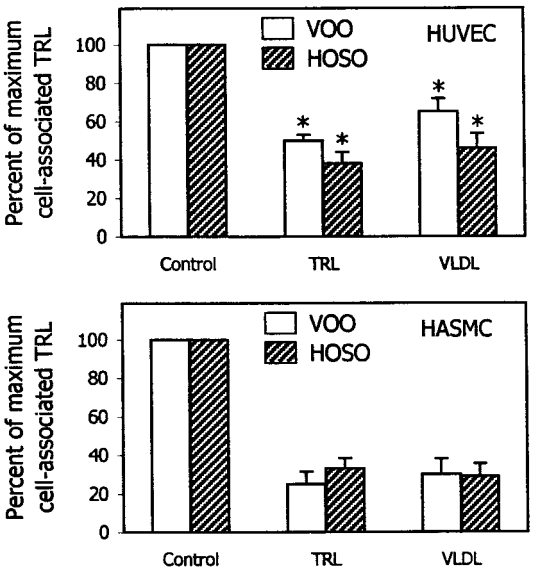

Figure 5. Competition cell association of native apolipoprotein E-containing lipoproteins (TRL and VLDL) and labeled TRL to vascular cells. Cells were cultured in 96 MicroWell flat bottom plates and incubated with 50 μg of DiI-TRL triacylglycerol/mL for 2 h at 37 °C in the absence or in the presence of 5-fold excess of unlabeled TRL and VLDL as indicated. Fluorescence was measured as described in Figure 2. Values are expressed as percent of maximum cell-associated TRL which were 42 ± 2.9 or 27 ± 1.8 μg of TG/mg of cell protein for DiI-TRL from VOO or HOSO in HUVEC, and 14 ± 1.1 or 8 ± 0.8 μg of TG/mg of cell protein for DiI-TRL from VOO or HOSO in HASMC. Results are means ± SD of eight wells. Asterisk means significant ($P < 0.05$) differences between VOO and HOSO.

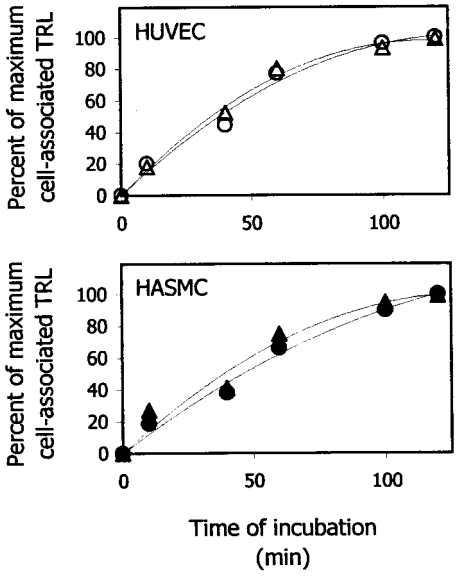

Figure 6. Time-response measurement of cell-associated DiI-TRL in vascular cells. Cells were cultured in 96 MicroWell flat bottom plates and incubated with 50 μg of DiI-TRL triacylglycerol/mL for 0−120 min at 37 °C. Fluorescence was measured as described in Figure 2. Values are expressed as percent of maximum cell-associated TRL, which were 44 ± 3.4 or 26 ± 1.9 μg of TG/mg of cell protein for DiI-TRL from VOO (open circles) or HOSO (open triangles) in HUVEC, and 14 ± 1.3 or 8 ± 0.6 μg of TG/mg of cell protein for DiI-TRL from VOO (filled circles) or HOSO (filled triangles) in HASMC. Each point represents the mean of eight wells which varied by less than 10%.

the origin/lipid composition of the ligand. It is unlikely that the labeling of TRL with DiI could physically exert

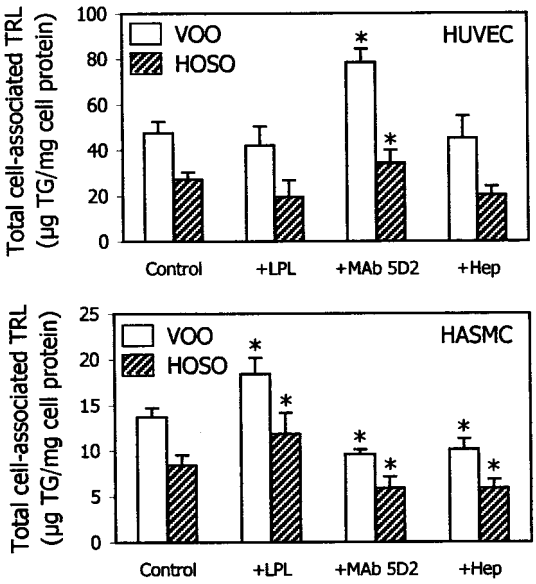

Figure 7. Effect of LPL, monoclonal antibody 5D2, or heparin to cell association of DiI-TRL in vascular cells. Cells were cultured in 96 MicroWell flat bottom plates and incubated with 50 μg of DiI-TRL triacylglycerol/mL for 2 h at 37 °C. Cells were pretreated with purified LPL (5 μg/mL), MAb 5D2 (5 μg/mL), or heparin (10 U/mL) as indicated. Fluorescence was measured as described in Figure 2. Results are means ± SD of eight wells. Asterisk means significant ($P < 0.05$) differences between VOO or HOSO and control.

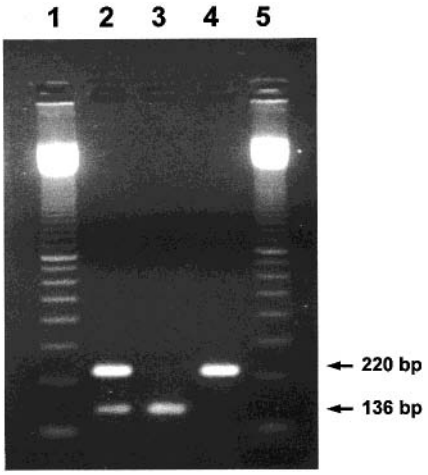

Figure 8. Expression of the VLDL receptor in HASMC. RT-PCR analysis was performed using exon 16 flanking primers (WBV-1 and WBV-17). Clones #21 (lane 4) and #7 (lane 3) of the MDCK cell line from kidney of dog were used as controls for glycosylated and nonglycosylated isoforms of VLDLr that produced bands of 220 and 136 bp, respectively. PCR products were resolved on 2% agarose gel. Lines 1 and 5, molecular weight markers (100 bp fragments); lane 2, HASMC sample.

a cell type-dependent interaction since the labeling efficiency did not match with the apparent K_m values. Furthermore, the kinetics were the same for both, TRL and cell lines. Identical patterns of cell surface distribution for DiI-TRL and unlabeled TRL have been previously demonstrated in epithelial and mesenchymal cells (*37*, *39*), indicating that DiI may not influence the cell-binding properties of labeled lipoproteins.

We consider of major importance the selective response of HUVEC and HASMC to TRL from VOO. The rate of clearance of TRL has been proposed to be a key factor in the pathogenesis of atherosclerosis, probably due to their effects on the functionality of the wall of

the arteries (*1*−*4*). In the presence of LPL, cholesterol-rich lipoproteins are greatly bound to endothelial cell matrix by positively charged clusters of arginine and lysine domains of apolipoprotein B (*40*). However, our observations indicate that bovine LPL and heparin had no effect on the reactivity of triacylglycerol-rich lipoproteins with HUVEC, and rather we found a marked increase of cell association with TRL from VOO when HUVEC were preincubated with the monoclonal antibody 5D2. This immunological reagent is able to differentiate between monomeric inactive and dimeric active LPL, inhibits LPL enzyme activity, and binds to C-terminal LPL sequences involved in interaction with plasma lipoproteins and lipoprotein receptors (*41*). The highly immunoreactive loop of the folding domain of LPL is a lipid binding site. Therefore, the possibility that MAb 5D2 could promote the formation of a lipoprotein/immunoglobulin complex of cellular recognition and/or to the arrangement of the cell surface of HUVEC for TRL interaction cannot be ruled out. According to the competition studies, it should be noted that VLDLr was involved in the catabolism of TRL, yet there was a significant nonspecific component. Interestingly, a recent study has described the cloning and expression of a new receptor that specifically binds to apolipoprotein B-48 in reticuloendothelial cells (*42*) and could be a putative surface element for discriminating lipoproteins carrying apolipoproteins B or E. Our findings are consistent with the hypothesis of two different mechanisms for the uptake of TRL by HUVEC, both acting in a lipid-dependent fashion and not mediated by exogenous LPL or LPL-binding molecules. One of them is probably mediated through the VLDLr variant lacking the O-linked sugar domain. To keep in mind, the constitutive expression of a new member of the lipase gene family has been recently discovered in HUVEC (*43*). This novel "endothelial lipase" has high phosholipase activity but low triacylglycerol lipase activity, which are independent of external factors (i.e., apolipoprotein C-II, or any other serum factor). Whether such endogenous lipase participates in the recognition and uptake of TRL has to be elucidated. However, these and our data may suggest that HUVEC are more likely to be involved in nonremnant TRL catabolism. Furthermore, the *O*-glycosylation of the VLDLr does not establish its specificity for the binding of proteins (*35*), but nothing is known about the specificity of ligands rich in lipid components. Among VLDLr-expressing tissues, endothelial cells present higher levels of VLDLr than the neighboring parenchymal cells (*10*), indicating a critical function in the uptake and trans-endothelial transport of plasma lipoproteins. This acquires special interest as postprandial lipemia is a dynamic state persisting for most of the day, and the nature of dietary fats appears as a determinant for cellular TRL catabolism. In this regard, recent studies have shown that VOO promotes the formation of postprandial TRL that are more rapidly cleared from blood than TRL from HOSO in humans (*22*, *24*).

Contrary to HUVEC, our findings in HASMC demonstrated properties that are characteristic of the classical pathway for lipoproteins uptake organized by LPL-binding sites and direct HSP-mediated internalization. In the presence of purified LPL, TRL from VOO were better uptaken by HASMC than TRL from HOSO, suggesting a role for HASMC in the catabolism of remnant TRL. Heparin and MAb 5D2 had similar effects in partially reducing TRL uptake of control cells. It appeared that the VLDLr was the most important component in HASMC for the cell processing of postprandial TRL, with no significant differences between TRL from VOO or HOSO. HASMC expressed the two variants of the clustered O-linked sugar domain of the VLDLr, predominating the *O*-glycosylated isoform which gives stability to the receptor by blocking the access to protease-sensitive site(s) (*36*). The VLDLr, together with the apolipoprotein E receptor-2, are the only members of the LDLr supergene family that have been shown to have the spliced and nonspliced variants (*44*). However, the physiological significance for this posttranscriptional receptor differential splicing is not known. Our findings indicate that VLDLr isoforms have cell-specific expression with a different role for lipid metabolism, appearing the O-glycosylation of the ectodomain as involved in TRL recognition by HASMC. Accordingly, it has been recently shown that O-linked glycans can recognize sphingolipid- and cholesterol-rich membrane rafts in ligand-sorting studies with human intestinal cells (*45*).

In summary, postprandial TRL from two monounsaturated oils were differentially uptaken by cell lines representing vascular wall cells. It potentially implies selective physiologic effects of VOO and HOSO on blood vessels by providing cells with lipid metabolites differing in quality and quantity. Both oils had similar amount of oleic acid but differed in other minor fatty acids and triacylglycerol composition. We cannot exclude the possibility that the different lipid composition found in TRL after oil intake could have influenced their metabolic fate, including some effects on LPL and/or VLDLr activities. Our results provide, for the first time, evidence for the interaction of TRL with human vascular cells in a lipid-dependent fashion and suggest different receptor pathways in the postprandial lipoprotein uptake mechanistically linked to the cell type.

ABBREVIATIONS USED

DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-indocarbocyanide perchlorate; HASMC, human aorta smooth muscle cells; HOSO, high-oleic sunflower oil; HSP, heparan sulfate proteoglycans; HUVEC, human umbilical vein endothelial cells; LPL, lipoprotein lipase; TG, triacylglycerols; TRL, triacylglycerol-rich lipoproteins; VLDL, very low density lipoproteins; VLDLr, VLDL receptor; VOO, virgin olive oil.

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LITERATURE CITED

(1) Aengevaeren, W. R. Beyond lipids − the role of the endothelium in coronary artery disease. *Atherosclerosis* **1999**, 147, S11−S16.
(2) Weissberg, P. Mechanisms modifying atherosclerotic disease − from lipids to vascular biology. *Atherosclerosis* **1999**, 147, S3−S10.
(3) Celermajer, D. S. Endothelial dysfunction: does it matter? Is it reversible?. *J. Am. Coll. Cardiol.* **1997**, 30, 325−333.
(4) Ross, R. Atherosclerosis − an inflammatory disease. *N. Eng. J. Med.* **1999**, 340, 115−126.
(5) Hodis, N. H. Pathophysiology of triglyceride-rich lipoproteins in atherothrombosis: clinical aspects. *Clin. Cardiol.* **1999**, 22, II15−II20.

(6) Hodis, N. H. Triglyceride-rich lipoprotein remnant particles and risk of atherosclerosis. *Circulation* **1999**, 99, 2852−2854.
(7) Cohn, J. S. Postprandial lipemia: emerging evidence for atherogenicity of remnant lipoproteins. *Can. J. Cardiol.* **1998**, 14, 18B-27B.
(8) Zilversmith, D. B. Atherogenic nature of triglycerides, postprandial lipidemia, and triglyceride-rich remnant lipoproteins. *Clin. Chem.* **1995**, 41, 153−158.
(9) Niemeier, A.; Gåfvels, M.; Heeren, J.; Meyer, N.; Angelin, B.; Beisiegel, U. VLDL receptor mediates the uptake of human chylomicron remnants in vitro. *J. Lipid Res.* **1996**, 37, 1733−1742.
(10) Wyne, K. L.; Pathak, R. K.; Seabra, M. C.; Hobbs, H. H. Expression of the VLDL receptor in endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* **1996**, 16, 407−415.
(11) Multhaupt, H. A.; Gåfvels, M. E.; Kariko, K.; Jin, H.; Arenas-Elliott, C.; Goldman, B. I.; Strauss, J. F.; Angelin, B.; Warhol, M. J.; McRae, K. R. Expression of very low-density lipoprotein (VLDL) receptor in the vascular wall: analysis of human tissues by in situ hybridization and immunohistochemistry. *Am. J. Pathol.* **1996**, 148, 1985−1997.
(12) Gåfvels, M. E.; Caird, M.; Britt, D.; Jackson, C. L.; Patterson, D.; Strauss, J. F. Cloning of a cDNA encoding a putative human very low-density lipoprotein/apolipoprotein E receptor and assigment of the gene to chromosome 9pter-023. *Somat. Cell Mol. Genet.* **1993**, 19, 557−569.
(13) Goldberg, I. J. Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. *J. Lipid Res.* **1996**, 37, 693−707.
(14) Argraves, K. M.; Battey, F. D.; MacCalman, C. D.; McCrae, K. R.; Gåfvels, M.; Kozarsky, K. F.; Chappell, D. A.; Strauss, J. F.; Strickland, D. K. The very low-density lipoprotein receptor mediates the cellular catabolism of lipoprotein lipase and urokinase-plasminogen activator inhibitor type I complexes. *J. Biol. Chem.* **1995**, 270, 26550−26557.
(15) Saxena, U.; Klein, M. G.; Goldberg, I. J. Metabolism of endothelial cell-bound lipoprotein lipase. Evidence for heparan sulfate proteoglycan-mediated internalization and recycling. *J. Biol. Chem.* **1990**, 22, 12880−12886.
(16) Weaver, A. M.; Lysiak, J. J.; Gonias, S. L. LDL receptor family dependent and -independent pathways for the internalization and digestion of lipoprotein lipase-associated β-VLDL by rat vascular smooth muscle cells. *J. Lipid Res.* **1997**, 38, 1841−1850.
(17) Karpe, F.; de Faire, U.; Mercuri, M.; Bond, M. G.; Hellenius, M. L.; Hamsten, A. Magnitude of alimentary lipemia is related to intima-media thickness of the common carotid artery in middle-aged men. *Atherosclerosis* **1998**, 141, 307−314.
(18) Karpe, F.; Hellenius, M. L.; Hamsten, A. Differences in postprandial concentrations of very-low-density lipoprotein and chylomicron remnants between normotriglyceridemic and hypertriglyceridemic men with and without coronary heart disease. *Metabolism* **1999**, 48, 301−307.
(19) Marcoux, C.; Hopkins, P. N.; Wang, T.; Leary, E. T.; Nakajima, K.; Davignon, J.; Cohn, J. S. Remnant-like particle cholesterol and triglyceride levels of hypertriglyceridemic patients in the fed and fasted state. *J. Lipid Res.* **2000**, 41, 1428−1436.
(20) Roche, H. M.; Zampelas, A.; Jackson, K. G.; Williams, C. M.; Gibney, M. J. The effect of test meal monounsaturated fatty acid: saturated fatty acid ratio on postprandial lipid metabolism. *Br. J. Nutr.* **1998**, 79, 419−424.
(21) Roche, H. M.; Gibney, M. J. Effect of long-chain n-3 polyunsaturated fatty acids on fasting and postprandial triacylglycerol metabolism. *Am. J. Clin. Nutr.* **2000**, 71, S232−S237.
(22) Abia, R.; Pacheco, Y. M.; Perona, J. S.; Montero, E.; Muriana, F. J. G.; Ruiz-Gutierrez, V. The metabolic availability of dietary triacylglycerols from two high oleic oils during the postprandial period does not depend on the amount of oleic acid ingested by healthy men. *J. Nutr.* **2001**, 131, 59−65.
(23) Goldberg, I. J.; Kako, Y.; Lutz, E. P. Responses to eating: lipoproteins, lipolytic products and atherosclerosis. *Curr. Opin. Lipidol.* **2000**, 11, 235−241.
(24) Abia, R.; Perona, J. S.; Pacheco, Y. M.; Montero, E.; Muriana, F. J. G.; Ruiz-Gutierrez, V. Postprandial triacylglycerols from dietary virgin olive oil are selectively cleared in humans. *J. Nutr.* **1999**, 129, 2184−2191.
(25) Ruiz-Gutierrez, V.; Muriana, F. J. G.; Guerrero, A.; Cert, A. M.; Villar, J. Plasma lipids, erythrocyte membrane lipids and blood pressure of hypertensive women after ingestion of dietary oleic acid from two different sources. *J. Hypertens.* **1996**, 14: 1483−1489.
(26) Ruiz-Gutierrez, V.; Muriana, F. J. G.; Guerrero, A.; Villar, J. Olive oil and high-oleic sunflower oil on human plasma and erythrocyte membrane lipids. *Nutr. Res.* **1997**, 17: 1391−1399.
(27) Ruiz-Gutierrez, V.; Morgado, N.; Prada, J. L.; Perez-Jimenez, F.; Muriana, F. J. G. Composition of human VLDL triacylglycerols after ingestion of olive oil and high-oleic sunflower oil. *J. Nutr.* **1998**, 128, 570−576.
(28) Ruiz-Gutierrez, V.; Perona, J. S.; Pacheco, Y. M.; Muriana, F. J. G.; Villar, J. Incorporation of dietary triacylglycerols from olive oil and high-oleic sunflower oil into VLDL triacylglycerols of hypertensive patients. *Eur. J. Clin. Nutr.* **1999**, 53, 687−693.
(29) Kotite, L.; Bergeron, N.; Havel, R. J. Quantification of apolipoproteins B-100, B-48, and E in human triglyceride-rich lipoproteins. *J. Lipid Res.* **1995**, 36, 890−900.
(30) Mills, G. L.; Lane, P. A.; Week, P. K. Laboratory Techniques in Biochemistry and Molecular Biology. In A Guidebook to Lipoprotein Technique; Burdon, R. H., Van Knippenrbergh, P. H., Eds.; Elsevier Science Publishing: Amsterdam, The Netherlands, 1989.
(31) Stephan, Z. F.; Yurachek, E. C. Rapid fluorometric assay of LDL receptor activity by DiI-labeled LDL. *J. Lipid Res.* **1993**, 34, 325−330.
(32) Saxena, U.; Witte, L. D.; Goldberg, I. J. Release of endothelial cell lipoprotein lipase by plasma lipoproteins and free fatty acids. *J. Biol. Chem.* **1989**, 264, 4349−4355.
(33) Teupser, D.; Thiery, J.; Walli, A. K.; Seidel, D. Determination of LDL-and scavenger-receptor activity in adherent and nonadherent cultured cells with a new single-step fluorometric assay. *Biochim. Biophys. Acta* **1996**, 1303, 193−198.
(34) Perona, J.; Barron, L. J.; Ruiz-Gutierrez, V. Determination of rat liver triglycerides by gas−liquid chromatography and reversed-phase high-performance liquid chromatography. *J. Chromatogr.* **1998**, 706, 173−179.
(35) Magrané, J.; Reina, M.; Pagan, R.; Luna, A.; Casaroli-Marano, R. P.; Angelin, B.; Gåfvels, M.; Vilaró S. Bovine aortic endothelial cells express a variant of the very low-density lipoprotein receptor that lacks the O-linked sugar domain. *J. Lipid Res.* **1998**, 39, 2172−2181.
(36) Magrané, J.; Casaroli-Marano, R. P.; Reina, M.; Gåfvels, M.; Vilaró S. The role of O-linked sugars in determining the very low-density lipoprotein receptor stability or release from the cell. *FEBS Lett.* **1999**, 451, 56−62.
(37) Casaroli-Marano, R. P.; Garcia, R.; Vilella, E.; Olivecrona, G.; Reina, M.; Vilaro, S. Binding and intracellular trafficking of lipoprotein lipase and triacylglycerol-rich lipoproteins by liver cells. *J. Lipid Res.* **1998**, 39, 789−806.
(38) Barak, L. S.; Webb, W. W. Fluorescent low-density lipoprotein for observation of dynamics of individual receptor complexes on cultured human fibroblasts. *J. Cell. Biol.* **1981**, 90, 595−604.

(39) Fernandez-Borja, M.; Bellido, D.; Vilella, E.; Olivecrona, G.; Vilaro, S. Lipoprotein lipase-mediated uptake of lipoprotein in human fibroblasts: evidence for an LDL receptor-independent internalization pathways. *J. Lipid Res.* **1996**, 37, 464−481.

(40) Saxena, U.; Auerbach, B. J.; Ferguson, E.; Wolle, J.; Marcel, Y. L.; Weisgraber, K. H.; Hegele, R. A.; Bisgaier, C. L. Apolipoprotein B and E basic amino acid clusters influence low-density lipoprotein association with lipoprotein lipase anchored to endothelial cell matrix. *Arterioscler. Thromb. Vasc. Biol.* **1995**, 15, 1240−1247.

(41) Chang, S. F.; Reich, B.; Brunzell, J. D.; Will, H. Detailed characterization of the binding site of the lipoprotein lipase-specific monoclonal antibody 5D2. *J. Lipid Res.* **1998**, 39, 2350−2359.

(42) Brown, M. L.; Ramprasad, M. P.; Umeda, P. K.; Tanaka, A.; Kobayashi, Y.; Watanabe, T.; Shimoyamada, H.; Kuo, W. L.; Li, R.; Song, R.; Bradley, W. A.; Gianturco, S. H. A macrophage receptor for apolipoprotein B48: cloning, expression, and atherosclerosis. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, 97, 7488−7493.

(43) Rader, D. J.; Jaye, M. Endothelial lipase: a new member of the triglyceride lipase gene family. *Curr. Opin. Lipidol.* **2000**, 11: 141−147.

(44) Sun, X. M.; Soutar, A. K. Expression in vitro of alternatively spliced variants of the messenger RNA for human apolipoprotein E receptor-2 identified in human tissues by ribonuclease protection assays. *Eur. J. Biochem.* **1999**, 262, 230−239.

(45) Alfalah, M.; Jacob, R.; Preuss, U.; Zimmer, K. P.; Naim, H.; Naim, H. Y. O-linked glycans mediate apical sorting of human intestinal sucrase-isomaltase through association with lipid rafts. *Curr. Biol.* **1999**, *9*, 593−596.

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