Triglyceride-Rich Lipoprotein Regulates APOB48 Receptor Gene Expression in Human THP-1 Monocytes and Macrophages1–3

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Abstract
The postprandial metabolism of dietary fats implies that the production of TG-rich lipoproteins (TRL) contributes to the progression of plaque development. TRL and their remnants cause rapid receptor-mediated monocyte/macrophage lipid engorgement via the cell surface apoB48 receptor (apoB48R). However, the mechanistic basis for apoB48 receptor (APOB48R) regulation by postprandial TRL in monocytes and macrophages is not well established. In this study, we investigated the effects of postprandial TRL from healthy volunteers on the expression of APOB48R mRNA and lipid uptake in human THP-1 monocytes and THP-1–derived macrophages. The expression of APOB48R mRNA was upregulated in THP-1 monocytes, but downregulated in THP-1–derived macrophages when treated with postprandial TRL (P < 0.05), in a dose- and time-dependent manner. TG and free cholesterol were dramatically increased in THP-1–derived macrophages (140 and 50%, respectively; P < 0.05) and in THP-1 monocytes (160 and 95%, respectively; P < 0.05). This lipid accumulation was severely decreased (~50%; P < 0.05) in THP-1–derived macrophages by small interfering RNA (siRNA) targeting of APOB48R. Using PPAR and retinoid X receptor (RXR) agonists, antagonists, and siRNA, our data indicate that PPARa, PPARγ, and RXRa are involved in postprandial TRL-induced APOB48R transcriptional regulation. Co-incubation with acyl-CoA synthetase or acyl-CoA:cholesterol acyltransferase inhibitors potentiated the effects of postprandial TRL on the expression of APOB48R mRNA in THP-1 monocytes and THP-1–derived macrophages. Our findings collectively suggest that APOB48R represents a molecular target of postprandial TRL via PPAR-dependent pathways in human THP-1 monocytes and macrophages and advance a potentially important link between postprandial metabolism of dietary fats and atherogenesis. J. Nutr. 142: 227–232, 2012.

Introduction
Growing evidence suggests that postprandial hypertriglyceridemia contributes to the pathogenesis of atherosclerosis, including coronary heart disease, stroke, and subsequent sudden death (1,2). In the postprandial state, the lipid moieties in the circulation exist mainly in the form of TRL8 carrying one

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3Supplemental Figure 1 is available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
4B.B. and S.L. contributed equally to this paper.
5Abbreviations used: ACAT, acyl-CoA:cholesterol acyltransferase; apoB48R, apoB48 receptor; CE, cholesteryl ester; 9cRA, 9-cis-retinoic acid; FC, free cholesterol; HPRT, hypoxanthine phosphoribosyltransferase; PMA, phorbol 12-myristate-13-acetate; RPLP0, ribosomal protein large P0; RXR, retinoid X receptor; siRNA, small interfering RNA; TRL, TG-rich lipoprotein.
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effect of pitavastatin in suppressing macrophage-derived foam cell functions through the regulation of \textit{APOB48R} (8).

Postprandial TRL circulate together with monocytes and can enter the arterial intima, even becoming trapped preferentially over LDL (9). However, studies on how postprandial TRL affect the molecular regulation and functional properties of \textit{APOB48R} in monocytes and mature macrophages have not, to our knowledge, been conducted. Therefore, we set out to examine whether postprandial TRL could influence the transcriptional regulation of \textit{APOB48R} in THP-1 monocytes and THP-1-derived macrophages and whether this could mediate lipid uptake from postprandial TRL.

\section*{Methods}

\subsection*{Preparation and characterization of postprandial TRL and fasting VLDL from healthy volunteers.}

This study was conducted according to the guidelines of good clinical practice. Prior to the beginning of the study, all participants provided their informed consent using protocols approved by the Human Clinical Commission and Ethics Committee of University Hospital Virgen del Rocio, Seville. The investigation conformed with the principles outlined in the Helsinki Declaration of the World Medical Association.

Fasting blood samples from six healthy donors were taken 12 h after the evening meal. The participants were then administered a high-fat meal consisting of butter (50 g/m² body surface area) along with a portion of plain pasta, one slice of brown bread, and one skim-milk yogurt (10). Blood was drawn 2–3 h following the high-fat meal ingestion, a time point previously shown to correlate with the most recently (nascent) secreted particles by the intestine. ApoB48-containing TRL were isolated from plasma by ultracentrifugation (11), pooled, dialyzed against PBS, and immediately stored at 8°C. Only once-thawed, postprandial TRL samples were used. Plasma TG were measured on a Hitachi Modular Analytics D-2400 analyzer using commercially available reagents and an enzyme-based kit (TG GPO-PAP, Roche Diagnostics). Total TG and cholesterol in postprandial TRL were measured using enzyme-based colorimetric kits (TG GPO and Infinity Cholesterol, Thermo Trace). The means of ratio TG:cholesterol was 2.5. ApoB100 and apoB48 were separated by SDS-PAGE and scanned with a laser densitometer. Mean postprandial TRL apoB48 was 5-fold higher relative to apoB100. We therefore considered postprandial TRL as large, apoB48-rich lipoproteins. Lipid oxidizability of postprandial TRL was checked (TBARS level) during isolation and storage, but oxidation of lipids was not detected (62 ± 27 nmol/mmol TG). VLDL were prepared by sequential ultracentrifugation (12).

\subsection*{Cell culture and treatments.}

The human monocytic THP-1 cell line, in suspension, was cultured in RPMI medium 1640 supplemented with l-glutamine, penicillin, streptomycin, and 10% heat-inactivated FBS. Monocytes were differentiated at a density of 7 × 10^5 cells/L with PMA (100 nmol/L, Sigma) for 4 d. Non-PMA-stimulated THP-1 cells (referred to as THP-1 monocytes) or PMA-stimulated THP-1 cells (referred to as THP-1-derived macrophages) were exposed to RPMI medium containing postprandial TRL, at different concentrations and times, along with or without PPARα antagonist MK886, PPARγ agonist GW9662, PPARα agonist Wy14643, PPARγ agonist 15-d-PGJ2, RXR agonist 9cRA, RXRα-heterodimer transactivation inhibitor As_{2}O_{3}, acyl-CoA synthetase inhibitor Triacsin D (kindly provided by Dr. Satoshi Omura, The Kitasato Institute, Japan), or ACAT inhibitor 58–035 (a generous gift from Novartis). All reagents were from Sigma except as indicated.

\subsection*{Intracellular lipid analysis.}

Total cellular lipids were extracted by using a hexane/isopropl alcohol (3:2) mixture, followed by cellular protein extraction with 0.2 mol/L NaOH. TG were determined using a TG GOP-PAP kit (Roche Diagnostics). GC and CE were determined by GC-flame ionization detector. Methyl heptadecanoic ester and α-cholestanol were used as internal standards. Cellular FFA levels were extracted (13) and quantified using a NEFA-HR kit (Wako Chemicals). Hematoxylin and Oil red O were used for nuclei and intracellular neutral lipid staining, and the stained cells were examined by using an Olympus model IX81 inverted phase microscope fitted with a DP71 Olympus digital camera. Cell R software (Olympus) was used for acquisition and further image analysis.

\subsection*{mRNA extraction and analysis of PCR products.}

Total RNA was extracted from THP-1 monocytes and THP-1-derived macrophages by using Trizol Reagent (Roche). RNA quality was assessed using the OD_{260}/OD_{280} ratio in a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific). RNA (2 μg) was subjected to RT at 42°C for 1 h. The reaction mixture contained 1 × RT buffer, 1 mmol/L deoxynucleotide triphosphate mix, 20 units of ribonuclease inhibitor, and 200 units of revertAid M-MuLV reverse transcriptase (Fermentas). One-tenth (2 μL) of the resulting cDNA was used as a template for qRT-PCR. The mRNA levels for specific genes were determined in a MX3000P system (Stratagene). For each PCR, a cDNA template was used to Brilliant SYBR green QPCR Master mix (Stratagene) containing the primer pairs for \textit{APOB48R}, PPARα, PPARγ, RXRα, or for RPLP0 and HPRT as housekeeping genes. The sequence and information for primers used in this study are as follows: \textit{APOB48R} (GenBank accession no. NM_182804); 5′-GCCAGTGACTCCTCTTCCTCTG-3′ (forward) and 5′-AGGATGCCACAGACTGACTCCTG-3′ (reverse); PPARα (NM_001001928); 5′-GTGGTAAAGGGGTAAACGCAAAC-3′ and 5′-GCTAATCGAGAGGGTTGAGG-3′; PPARγ (NM_138712); 5′-GCGTGAGAGATCACAGACA-3′ and 5′-GGGTTCAATATAGCTACACAA-3′; RXRα (NM_002957); 5′-GGTGTCTTCCCTCTTGGAGG-3′ and 5′-GGTTGTTGCTTCCCTTGGAC-3′; HPRT (NM_001001928); 5′-ACCCGCGAAGATTGTGTGAGA-3′ and 5′-AAGCATGTTGCCACAGACCT-3′. All amplification reactions were performed in triplicate. The magnitude of change of mRNA expression for candidate genes was calculated by using the standard 2^{-ΔΔCt} method. All data were normalized to endogenous reference (RPLP0 and HPRT) gene levels and expressed as percentage of control.

\subsection*{siRNA transfections.}

siRNA derived from human target sequences were synthesized by Dharmacon Research using SMARTpool selection to suppress the expression of \textit{APOB48R}, PPARα, PPARγ, and RXRα. Cells were transfected with siRNA (100 nmol/L) by using Oligofectamine from Invitrogen. The efficiency for siRNA delivery was tested with siTOX Transfection Control (Dharmacon Research). We also used siCONTROL Non-Targeting siRNA Pool for negative control siRNA and siCONTROL GAPDH siRNA for positive control siRNA (Dharmacon Research). Two days after transfection, the cells were treated with postprandial TRL.

\subsection*{Statistical analysis.}

Data are presented as mean ± SD. The homogeneity of variance was tested with Bartlett’s test. Comparisons of two means were performed by using Student’s t test. Group statistical comparisons were performed by 1- or 2-way ANOVA with a post hoc Bonferroni test. A value of P < 0.05 was considered different. All experiments were performed at least three times in triplicate.

\section*{Results}

\textit{APOB48R} mRNA in THP-1 monocytes and THP-1-derived macrophages. The expression of \textit{APOB48R} mRNA was similar in THP-1 monocytes (3410 ± 240 \textit{APOB48R} mRNA copies; n = 3) and THP-1-derived macrophages (2770 ± 550 \textit{APOB48R} mRNA copies; n = 3). ApoB48 protein expression was not analyzed, because there is no commercially antibody available.

\textit{APOB48R} mRNA is increased in THP-1 monocytes but decreased in THP-1-derived macrophages by human postprandial TRL. The expression of \textit{APOB48R} mRNA was significantly increased in THP-1 monocytes (Fig. 1A) but...
decreased in THP-1–derived macrophages (Fig. 1C) relative to nonloaded control cells. These effects occurred in a dose- (Fig. 1A,C) and time- (Fig. 1B,D) dependent manner and were not altered by the presence of VLDL (Fig. 2), indicating that apoB100-containing lipoproteins are not involved in postprandial TRL-mediated expression of APOB48R mRNA.

**Foam cell formation is modulated in THP-1 monocytes and THP-1–derived macrophages in an APOB48R-dependent manner by human postprandial TRL.** Oil Red O staining and quantitative lipid analysis showed a dramatic increase in neutral lipid droplets (mainly TG) (Supplemental Fig. 1; Fig. 3A) as well as a modest increase in CE and FC (Fig. 3C) after the incubation of THP-1–derived macrophages with postprandial TRL. Interestingly, these lipids also accumulated, but to a lesser extent, in THP-1 monocytes when incubated with postprandial TRL (Figs. 3A–C). To determine the possible role of APOB48R in foam cell formation, siRNA-mediated knockdown studies were undertaken. The expression of APOB48R mRNA was reduced by the APOB48R-siRNA in THP-1 monocytes (–73 ± 3%) (P < 0.05) and THP-1–derived macrophages (–78 ± 5%) (P < 0.05) compared to the control siRNA. The efficiency of siRNA uptake was >90%. Under these conditions, we found substantial decreases of TG (Fig. 3A), CE (Fig. 3B), and FC (Fig. 3C) in THP-1–derived macrophages, which was greater (P < 0.05) in the presence of postprandial TRL. Conversely, THP-1 monocytes with a similar reduction of expression of APOB48R mRNA (>70%) dramatically accumulated TG (Fig. 3A), CE (Fig. 3B), and FC (Fig. 3C).

**Involvement of PPARα, PPARγ, and RXRα in human postprandial TRL-dependent APOB48R mRNA in THP-1 monocytes and THP-1–derived macrophages.** Treatment of THP-1 monocytes and THP-1–derived macrophages with MK886 (PPARα antagonist) (14) or GW9662 (PPARγ antagonist) (15) resulted in dramatic changes in the expression of APOB48R mRNA after incubation with postprandial TRL (Table 1). The expression of APOB48R mRNA decreased in THP-1 monocytes and increased in THP-1–derived macro-

![FIGURE 2](https://academic.oup.com/jn/article-abstract/142/2/227/4743460)

**FIGURE 2** Transcriptional regulation of APOB48R in THP-1 cells treated with postprandial TRL for 12 h in the absence or presence of VLDL. Values are mean ± SD, n = 3. Means without a common letter differ, P < 0.05. apoB48R, apoB48 receptor; TRL, TG-rich lipoprotein.

**Discussion**

Accumulating evidence concerning TG levels in the postprandial state as a predictor of higher coronary heart disease and stroke
risk (1,2) suggests atherogenesis as a phenomenon in which apoB48-containing TRL could play an important role. According to the current paradigm, the atherosclerotic process is primarily initiated in the vascular wall itself by: 1) recruitment of circulating monocytes into atherogenic foci; 2) retention of these monocytes as monocyte-derived macrophages; and 3) transformation of macrophages to foam cells by accumulating lipids. However, unprocessed monocytes represent a large pool of circulating precursors that coexist in the bloodstream with postprandial TRL and may start accumulating lipids even prior to their migration to tissues and differentiation to macrophages. It is thought that apoB48R mediates the rapid, high-affinity uptake of postprandial TRL (4). However, the mechanism by which postprandial TRL influences the regulation and functional properties of apoB48R in monocytes and macrophages has not yet been studied.

Here, we show that postprandial TRL regulate apoB48R gene transcription in THP-1 monocytes and THP-1–derived macrophages. Our findings reveal that postprandial TRL have opposite effects, in a time- and dose-dependent manner, on the expression of apoB48R mRNA in THP-1 monocytes (upregulating) and THP-1–derived macrophages (downregulating). However, changes in apoB48R mRNA abundance might not reflect apoB48R protein abundance, because we could not analyze the protein levels of apoB48R. A positive-feedback loop that elevates apoB48R gene transcription could mediate postprandial TRL clearance by THP-1 monocytes, which is the mechanism that underlies the transcriptional activity of macrophage scavenger receptors in response to oxidized LDL (19). Thus, the expression of apoB48R mRNA in THP-1 monocytes may be perpetuated by a cycle in which postprandial TRL drives its own uptake. In contrast, a negative-feedback mechanism in which postprandial TRL repress apoB48R gene transcription could operate in THP-1–derived macrophages, as previously described for the monocyte LDL receptor in response to native LDL (20). However, the ability of postprandial TRL to promote cytoplasmic lipid accumulation was higher in THP-1–derived macrophages than in THP-1 monocytes, suggesting that apoB48R could be highly functional for binding and internalization of postprandial TRL at the monocyte differentiation stage.

Additionally, our results demonstrate that gene silencing of apoB48R by siRNA inhibits macrophage-derived formation of foam cells upon challenge with postprandial TRL, further implicating apoB48R as a potential major contributor in postprandial TRL-mediated atherosclerotic foam cell development. Our study also provides evidence that large apoB48-rich lipoproteins, as well as small, apoB48-poor, remnant-like

### Table 1: Transcriptional regulation of apoB48R in THP-1 cells treated with postprandial TRL (50 mg/L TG) for 12 h in the absence or presence of PPAR antagonists and agonists

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Control</th>
<th>MK866</th>
<th>GW9662</th>
<th>Wy14643</th>
<th>15-d-PGJ</th>
</tr>
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<tr>
<td>THP-1 monocytes</td>
<td>100a</td>
<td>b</td>
<td>c</td>
<td>d</td>
<td>e</td>
</tr>
<tr>
<td>THP-1–derived macrophages</td>
<td>100f</td>
<td>g</td>
<td>h</td>
<td>i</td>
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1 Values are mean ± SD, n = 3. Means in a row without a common letter differ, P < 0.05. MK866 (3 μmol/L), PPARa antagonist; GW9662 (20 μmol/L), PPARγ antagonist; Wy14643 (15 μmol/L), PPARa agonist; 15-d-PGJ (30 μmol/L), PPARγ agonist. apoB48R, apoB48 receptor; TRL, TG-rich lipoprotein.
lipoproteins (8), could be involved in atherogenic processes via apoB48R. The observation that postprandial TRL cause dramatic increases of lipid accumulation in APOB48R-siRNA–transfected THP-1 monocytes is unexpected. This probably suggests that endogenous lipid biosynthesis compensates for the deficiency of apoB48R in THP-1 monocytes, contributing to restore the supply of ligands (FFA) for nuclear receptors (21) and channel the excess FFA toward lipid stores (22). It can also be speculated that APOB48R could directly or indirectly function as a molecular break or desensitizer of mechanisms involved in the regulation of monocyte lipid accumulation, a condition that would be bypassed in macrophages.

Activators (Wy14643 and 15-d-PGJ2) of PPARα and PPARγ decrease the expression of APOB48R and lipid uptake in human THP-1 and peripheral, blood-borne, mature macrophages (6). We obtained confirmatory findings in THP-1–derived macrophages by using antagonists of PPARα (MK866) and PPARγ (GW9662) and siRNA oligonucleotides targeting PPARα and PPARγ coadministered with postprandial TRL. In an identical set of experiments in THP-1 monocytes, we reciprocally observed a decrease in the expression of APOB48R mRNA in THP-1 monocytes, an effect that was reversed by Wy14643 and 15-d-PGJ2. These data anticipate that distal transcriptional effects of postprandial TRL on APOB48R are tightly regulated by PPARα and PPARγ. These PPAR do not act alone but as a heterodimer with their obligate partner, RXRα (23). We used three alternative methods to establish that RXRα is a key element involved in the expression of APOB48R mRNA. Comparable to other nuclear receptors, the binding of ligand to PPAR stimulates the recruitment of co-regulators, and subsequent binding of the ligand-bound heterodimeric PPAR-RXR complex to DNA response elements in the promoter or enhancer regions of target genes (so-called direct repeat type I element or peroxisome proliferator responsive element) alters co-activator/co-repressor dynamics for their transcription (6,24). This dual transcriptional activity of PPAR enables them to both activate and inhibit gene expression. To the best of our knowledge, our study is the first to describe such effects on the APOB48R gene in a cell differentiation-dependent manner. Further studies to characterize the molecular mechanisms whereby postprandial TRL mediate APOB48R gene transactivation in monocytes and transrepression in macrophages will be required.

FFA can be intracellularly generated from postprandial TRL by enzymatic and nonenzymatic mechanisms (25), and they are the best-known, bone fide PPAR activators (17). Inside the cells, FFA are likely transported to the nucleus in association with fatty acid-binding proteins, which may function as positive regulators of PPARα and PPARγ (18). We observed that preventive reesterification of FFA released from lipolysis of postprandial TRL by inhibiting acyl-CoA synthetase or ACAT potentiated the effects of postprandial TRL on the expression of APOB48R mRNA in THP-1 monocytes and THP-1–derived macrophages, which further suggests the ability of postprandial TRL to modulate APOB48R gene transcription by increasing

![FIGURE 4](https://academic.oup.com/jn/article-abstract/142/2/227/4743460/FIGURE-4.png)

**FIGURE 4** Transcriptional regulation of APOB48R in THP-1 cells treated with postprandial TRL for 12 h in the absence or presence of RXRα agonist 9c-RA (A), RXRα inhibitor As2O3 (B), or RXRα-siRNA (100 nmol/L) (C). Values are mean ± SD, n = 3. Means without a common letter differ, P < 0.05. apoB48R, apoB48 receptor; RXR, retinoid X receptor; siRNA, small interfering RNA; TRL, TG-rich lipoprotein.
intracellular FFA concentration. Recent data have also linked intracellular fatty acid biosynthesis to the generation of physiologically relevant endogenous ligands for PPAR in the murine liver (26) and hypothalamus (27). Therefore, our observations contribute to the understanding of this issue and extend the influence of FFA derived from postprandial TRL, as PPAR ligands, on APOB48R gene expression to human monocytes.

In conclusion, we have elucidated the previously uncharacterized ability of postprandial TRL, in concert with PPARα/ PPARγ and RXRα, to modulate APOB48R gene expression in THP-1 monocytes and THP-1-derived macrophages. It is likely that apoB48R plays a primary role in postprandial TRL-mediated foam cell formation. Our experiments support the concept that postprandial TRL may function as a nutritional responsive entity that is chaperoned by apoB48R from the extracellular environment. Thus, APOB48R is potentially an intriguing target for the prevention of proatherogenic events during the postprandial state. This issue is especially relevant for the clinical outcomes of people who have pathologically exacerbated and delayed postprandial responses to the ingestion of dietary fats.

Acknowledgments

Literature Cited