Abstract

Postprandial triglyceride (TG)-rich lipoproteins (TRLs) transport dietary fatty acids through the circulatory system to satisfy the energy and structural needs of the tissues. However, fatty acids are also able to modulate gene expression and/or induce cell death. We investigated the underlying mechanism by which postprandial TRLs of different fatty acid compositions can induce cell death in human monocytes. Three types of dietary fat [refined olive oil (ROO), high-palmitic sunflower oil (HPSO), and butter] with progressively increasing SFA:MUFA ratios (0.18, 0.41, and 2.08, respectively) were used as a source of postprandial TRLs (TRL-ROO, TRL-HPSO, and TRL-BUTTER) from healthy men. The monocytic cell line THP-1 was used as a model for this study. We demonstrated that postprandial TRLs increased intracellular lipid accumulation (31–106%), reactive oxygen species production (268–349%), DNA damage (133–1467%), poly(ADP-ribose) polymerase 1 (800–1710%) and caspase-3 (696–1244%) activities, and phosphorylation of c-Jun NH2-terminal kinase (JNK) (54 kDa, 141–288%) and p38 (24–92%). These effects were significantly greater with TRL-BUTTER, and TRL-ROO did not induce DNA damage, DNA fragmentation, or p38 phosphorylation. In addition, blockade of p38, but not of JNK, significantly decreased intracellular lipid accumulation and increased cell death in postprandial TRL-treated cells. These results suggest that in human monocytes, p38 is involved in survival signaling pathways that protect against the lipid-mediated cytotoxicity induced by postprandial TRLs that are abundant in saturated fatty acids. J. Nutr. 143: 620–626, 2013.

Introduction

Recent studies have shown the potential effects of circulating TGs in postprandial TG-rich lipoproteins (TRLs) and artificial lipid emulsions to promote the in vitro and ex vivo activation of leukocytes, including T-cells, neutrophils, and monocytes (1–4). This early and frequently exaggerated TG-mediated leukocyte activation has been shown to result in the secretion of proinflammatory mediators that promote the infiltration of inflammatory cells and lipoproteins into the subendothelium, thus contributing to the development of atherosclerotic plaques (1, 5). Postprandial TRLs can also promote lipid accumulation in peripheral blood leukocytes prior to their inflammation-mediated recruitment (6), which is a feature of monocytes from patients with angiographic evidence of atherosclerosis (7). More recently, we reported that monocytes accumulate lipid droplets in an apoB48 receptor (apoB48R)-dependent manner during the course of a postprandial state in vivo (4). In vitro, we have also found that postprandial TRLs elicit cytotoxicity in monocytes, an effect that was dependent on the type of dietary fatty acid. Specifically, SFA-rich postprandial TRLs elicited more significant effects than MUFA-rich postprandial TRLs (8). In this study, we have extended our investigation to determine the procytotoxic signals and pathways by which the abundant SFA or MUFA amounts of postprandial TRLs induce cell death in human monocytes. Three different dietary fats [refined olive oil (ROO), high-palmitic sunflower oil (HPSO), and butter] were administered to healthy volunteers to obtain a panel of postprandial TRLs with increasing SFA:MUFA ratios.

Subjects and Methods

Subjects. Fourteen healthy, Caucasian, male nonsmokers (mean values ± SDs: BMI, 23.9 ± 1.9 kg/m²; age, 27 ± 7 y) participated in this study. The mean fasting total, LDL, and HDL cholesterol and plasma TG concentrations of the participants upon commencement of the screening were 4.09 ± 0.31, 2.34 ± 0.85, 1.39 ± 0.21, and 0.86 ± 0.27 mmol/L, respectively. More details on the participants and the study design were...
recently reported (9). Briefly, this was a randomized, crossover study that included a washout period of 1 wk of consumption of a National Cholesterol Education Program step I diet. Immediately after blood samples were acquired at 12 h after fasting, the participants consumed a fat-rich meal consisting of the corresponding dietary fats (ROO, HPSO, or butter at 50 g/m² body surface area). The ROO, HPSO, and butter were free of polyphenols as measured by reverse-phase HPLC-dioate array (10). The fatty acid composition of the meals was determined according to the methods previously described in EEC7796/2002 (11), and the SFA:MUFA ratios were as follows: 0.18, 0.41, and 2.08 for the ROO, HPSO, and butter meals, respectively. All of the protocols were approved by the Human Clinical Commission and the Ethics Committee of the Hospitales Universitarios Virgen del Rocio (SAS, Seville) prior to the start of the study and informed consent was obtained from each participant. The study conformed to the principles outlined in the Helsinki Declaration.

**TRL isolation.** The isolation of postprandial TRLs using a Svedberg flotation unit >400 from freshly postprandial plasma samples collected at 3 h after the consumption of the test meals was performed as previously described (12). The total TGs and cholesterol content in the postprandial TRLs were measured using enzyme-based colorimetric kits supplied by Thermo Trace (Triglycerides GPO and Infinity Cholesterol). To determine the fatty acid composition of the postprandial TRLs, we used a simple and rapid 1-step lipid extraction and FAME procedure according to the methods previously described by Garces and Mancha (13), with some modifications. The postprandial TRLs were pooled and stored at −70°C until further study.

**Reagents.** RPMI-1640 media, PBS, penicillin, streptomycin, t-glutamine, and FBS were purchased from Gibco (Life Technologies). 3-Aminobenzoamide (3-AB; 857734), bovine LDL (L2254), and Oil Red O (ORO) (O0625) were purchased from Sigma-Aldrich. Hoechst 33342 (H1399), H2DCFDA (D399), and Nile Red (N1142) were purchased from Molecular Probes (Life Technologies).

**Cell culture.** The human nonadherent monocytic THP-1 cell line (TIB-202; American Type Culture Collection) was cultured in RPMI-1640 medium supplemented with t-glutamine, penicillin, streptomycin, and 10% heat-inactivated FBS at 37°C in a humidified atmosphere of 5% CO₂ in air. Prior to the treatments, 3 × 10⁵ cells/L were incubated in medium containing 0.5% FBS for 24 h. The viability of the cells was determined by Trypan blue dye staining and counting using a hemocytometer (>95% of cells were viable). The cells were then stimulated with postprandial TRLs at a final concentration of 0.1 g TGs/L for an additional 48 h as previously described (8). The media were not changed during the 48-h incubations. The LPL treatments (2000 units/L) of the postprandial TRLs were administered for 30 min at 37°C. The total FFA content in cell culture media was measured using the Free Fatty Acid quantification kit obtained from Abcam (ab65341). The inhibitory concentration of FFAs in the cell culture media, which were 0.3 ± 0.1%, and 1.1 ± 0.1% in isopropyl alcohol for neutral lipids, counterstained with Mayer's hematoxylin, and examined by light microscopy. For nuclear morphology, the cells were stained with Hoechst 33342 and examined by fluorescence microscopy. The scoring of cells exhibiting condensed nuclei was performed using a minimum of 80–100 pictures per slide.

**Statistical analysis.** The results were expressed as the mean values ± SDs. Comparisons of the differences between these mean values were performed using a 1-way ANOVA. Tukey’s post hoc test was performed for multiple comparisons. For correlation analysis, the Spearman’s rho test was performed. A 2-way ANOVA was used to analyze the effect of SB202190, TRL-BUTTER, or the interaction between SB202190 and TRL-BUTTER. Western blot densities were measured using Labmage software (Kapelan). The data were analyzed using SPSS statistics version 17.0 for WINDOWS (IBM). P values <0.05 were considered significant.

**Results**

**Intracellular lipid accumulation.** After treatment of the human monocytes with TRL-ROO, TRL-HPSO, or TRL-BUTTER for 48 h, all of the postprandial TRLs induced intracellular lipid accumulation compared with the control treatment (P < 0.001) (Fig. 1A, B). The lipid accumulation was greater in the monocytes treated with TRL-BUTTER than in those treated with TRL-ROO or TRL-HPSO, which did not differ. Preincubation of the postprandial TRLs with LPL further promoted the accumulation of lipid droplets in the human monocytes (Fig. 1C). However, this treatment did not contribute to increase the concentration of FFAs in the cell culture media, which were 0.3 ± 0.1%, 0.4 ± 0.1%, and 1.1 ± 0.3% of the available esterified fatty acids in the TRL-ROO, TRL-HPSO, and TRL-BUTTER treated cells, respectively.

**Intracellular ROS production.** A flow cytometric analysis revealed an induction of intracellular ROS by postprandial TRLs for 48 h compared with the control treatment (P < 0.05), although no significant differences were detected between the types of TRLs (Fig. 2A).

**PARP-1 and caspase-3 activities.** The activities of both PARP-1 (Fig. 2B) and caspase-3 (Fig. 2C) were greater in monocytes...
treated with postprandial TRLs for 48 h compared with the control treatment and the effects of TRL-BUTTER were greater than those of TRL-ROO (P < 0.05). The increase in the SFA:MUFA ratios in the postprandial TRLs correlated with the increase in PARP-1 (r² = 0.95; P < 0.05) and caspase-3 (r² = 0.96; P < 0.05) activities, and a positive correlation was also determined between the activities of PARP-1 and caspase-3 (r² = 0.83; P < 0.05) in cells.

**PARP-1 cleavage and DNA damage.** By western-blot analysis, we observed an increase in PARP-1 cleavage in monocytes treated with postprandial TRLs for 48 h compared with control treatment (Fig. 3A,B). The effects of TRL-BUTTER and TRL-HPSO did not differ and were significantly greater than those of TRL-ROO. Inhibition of PARP-1 by 3-AB enhanced the postprandial TRL-induced PARP-1 cleavage (Fig. 3C,D). We also detected DNA damage (COMET assay) (Fig. 4A,B) and fragmentation (Fig. 5A,B) in the human monocytes after the TRL-BUTTER and TRL-HPSO treatments (P < 0.05).

**JNK and p38 phosphorylation.** A Western-blot analysis revealed an increase (P < 0.05) in the phosphorylation of the 54-kDa isoform of JNK in the cells treated with postprandial TRLs (TRL-BUTTER = TRL-HPSO > TRL-ROO) for 48 h compared with the control treatment (Fig. 6A,B). The amount of phosphorylated p38 was also greater with TRL-BUTTER than with TRL-ROO (P < 0.05) (Fig. 6C,D). When the cells were treated with postprandial TRLs in the presence of the JNK inhibitor SP600125, no further effect on cell death was observed (data not shown). However, the p38 inhibitor SB202190 markedly increased cell death (Fig. 7A) and decreased lipid accumulation (Fig. 7B) in the human monocytes treated with postprandial TRLs (all P < 0.05).

**Discussion**

Growing evidence indicates that high-fat meals can induce lipid accumulation, activation markers, cellular adhesion, and proinflammatory gene expression, which are common features of preactivation in peripheral leukocytes (1,4,17–19). Some of these postprandial TRL-mediated effects occur in monocytes in vivo (4,17) and in vitro (20) and require the involvement of LDL.
receptor related-protein (LRP1) and apoB48R. Furthermore, postprandial TRLs have the ability to perturb monocyte viability and cell cycle progression in a largely fatty acid-dependent manner (8). This study was designed to elucidate the underlying mechanisms by which postprandial TRLs (TRL-BUTTER > TRL-HPSO > TRL-ROO) render selective cytotoxic effects in human monocytes.

The existing information regarding the effect of dietary fatty acids on intracellular lipid accumulation in circulating leukocytes is limited. In this study, we show that the formation of lipid droplets increased in cells treated with TRL-BUTTER compared with TRL-ROO, suggesting that postprandial TRLs rich in the SFA palmitic acid, 16:0, mediate lipid accumulation in human monocytes. The relevance of the type of dietary fatty acids in the accumulated intracellular lipids is further supported by our observation of the gradual upregulation of apoB48R gene expression in human monocytes. This upregulation is attributed to the altered postprandial TRLs, which contain progressively increased ratios of 16:0 to MUFA oleic acid, 18:1n9 (L. M. Varela, A. Ortega, S. Lopez, R. Abia, F. J. G. Muriana, and B. Bermude, unpublished data). However, the intracellular lipid accumulation can also be triggered by the function of LPL. Apart from its bridging function, LPL can facilitate the hydrolysis of TG-containing lipoproteins as well as the uptake of FFAs. Our study demonstrates that LPL markedly increased the postprandial TRL-induced intracellular lipid accumulation, consistent with recent reports that lipolytic products generated from VLDLs can induce the formation of lipid-filled droplets within cultured THP-1 monocytes (21). These authors speculated that the sequestration of fatty acids into droplets could prevent them from initiating proapoptotic signals and/or generating ROS, 2 endpoints of lipotoxicity. Because 16:0 has been reported to be one of the most cytotoxic fatty acids in different cell systems (22,23), it is not surprising that postprandial TRLs from the most saturated fat (TRL-BUTTER) elicited the most significant extent of lipid droplet formation in human monocytes. Similar findings were reported regarding the induction of larger lipid droplets by synthetic 16:0 than by synthetic 18:1n9 (21).

Postprandial TRLs mediated an increase in the intracellular ROS levels in human monocytes that was comparable for any type of dietary fat in the meals. Previous studies also reported ROS production in human monocytes exposed to chylomicron remnants (24,25) and oxidized-LDL (26,27). Our data and studies have demonstrated the ability of postprandial TRLs to induce effects associated with increased oxidative stress in neutrophils but not in lymphocytes (1), suggesting that ROS...
production might represent a conserved response to postprandial TRLs throughout the myeloid cell lineage. However, unlike neutrophils (28) and monocyte-derived dendritic cells (29), which are better adapted to survive in highly oxidative environments, monocytes are not as resistant to oxidative stress. Supporting and extending our previous observations of postprandial TRL-induced necrosis and apoptosis in human monocytes, we now suggest that pathways mediated by PARP-1 and caspase-3 are likely involved in these processes. PARP-1 belongs to a group of nuclear enzymes that play a critical role in DNA damage repair (30). Increased PARP-1 activity is an energetically expensive process, resulting in failure of cellular ATP production, rapid depletion of NAD+, and eventual necrosis. Caspase-3 cleaves PARP-1 to prevent ATP depletion that would otherwise result from PARP-1 overactivation, promoting cells to undergo apoptosis (30,31). Our results demonstrated that postprandial TRLs promote cell death in human monocytes by increasing the 89-kDa, C-terminal fragment of PARP-1, an effect that was enhanced in the presence of DNA damage and the PARP-1 inhibitor 3-AB. We further observed that TRL-BUTTER elicited more cytotoxic signals than TRL-ROO, consistent with recent observations indicating that 16:0, but not 18:1n9, bound to albumin efficiently, promoting the de novo synthesis of the procytotoxic C16 ceramide in both early and mature human monocytes (32). These findings also suggest that the massive uptake of FFAs might exceed their ability to be incorporated into intracellular TGs or other neutral lipids (33).

Excessive ROS levels and PARP-1 or caspase-3 activities might indirectly damage cells by activating a variety of stress-sensitive, intracellular signaling pathways, such as those regulated by the MAPK family members JNK and p38 (34). For example, the increased expression and phosphorylation of p38 observed after a high-carbohydrate, high-fat meal challenge are suppressed if oxidative stress is neutralized in circulating mononuclear cells (35). FFAs have also been shown to induce phosphorylation as a prerequisite step for the activation of JNK and p38 in different cell types (33,36–38). However, the role of JNK and p38 in fatty acid-induced cytotoxicity remains unclear (30,33). To the best of our knowledge, this is the first report of JNK and p38 phosphorylation in human monocytes that are induced by postprandial TRLs in a fatty acid-dependent manner, with TRL-BUTTER eliciting the most significant effect. However, blockade of p38, but not of JNK, significantly decreased intracellular lipid accumulation and increased cell death, suggesting that p38 is likely involved in survival signaling pathways in human monocytes against lipid-mediated cytotoxicity of postprandial TRLs. This survival signal eventually occurs when p38 is chemically or genetically inhibited (39). Whereas this atypical cytoprotective role of p38 was not previously described in monocytes, a few studies have shown that the phosphorylation

![FIGURE 5](image-url) DNA fragmentation in human monocytes following treatment with TRL-ROO, TRL-HPSO, or TRL-BUTTER after 48 h. Untreated cells are referred to as control. Total DNA fragmentation was assessed by apoptotic bodies staining (A,B). Values shown in the graphs are the means ± SDs of n = 4 independent experiments. Means without a common letter differ, P < 0.05. HPSO, high-palmitic sunflower oil; ROO, refined olive oil; TRL, TG-rich lipoprotein.

![FIGURE 6](image-url) Phosphorylation of JNK (A,B) and p38 (C,D) in human monocytes following treatment with TRL-ROO, TRL-HPSO, or TRL-BUTTER for 48 h. Untreated cells are referred to as control. Equal loading of the proteins was confirmed by the tubulin (55 kDa) band. Values are the means ± SDs of n = 4 independent experiments. Within each graph, means without a common letter differ, P < 0.05. HPSO, high-palmitic sunflower oil; JNK, c-Jun NH2-terminal kinase; MW, molecular weight; ROO, refined olive oil; TRL, TG-rich lipoprotein.
of p38 mediates procytotoxic and survival signals depending on the cellular environment in macrophages and other cell types. For example, phosphorylated p38 elicits proapoptotic effects in cultured macrophages loaded with cholesterol (40) but antiapoptotic effects in the cholesterol-loaded plaque macrophages of mice (39). The cholesterol content in TRL-BUTTER was lower than that found in TRL-ROO (8); thus, a major role for cholesterol in postprandial TRL-induced cytotoxicity appears unlikely. An alternative explanation is that the bona fide cytoxic activation of JNK might represent one of the stressors that regulates the survival-death pathway that facilitates cytotoxicity (39). Nevertheless, our observations are consistent with the concept of a prominent role for p38 as a counterbalance of cell death pathways that rescue cells from the cytotoxic and steatogenic effects of postprandial TRLs rich in 16:0. These observations raise the possibility that meals containing 18:1n9 triglyceride-rich lipoproteins might be crucial for innate immune response.

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Literature Cited


