

# Dietary Oleic and Palmitic Acids Modulate the Ratio of Triacylglycerols to Cholesterol in Postprandial Triacylglycerol-Rich Lipoproteins in Men and Cell Viability and Cycling in Human Monocytes<sup>1-3</sup>

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## Abstract

The postprandial metabolism of dietary fats produces triacylglycerol (TG)-rich lipoproteins (TRL) that could interact with circulating cells. We investigated whether the ratios of oleic:palmitic acid and monounsaturated fatty acids (MUFA):SFA in the diet affect the ratio of TG:cholesterol (CHOL) in postprandial TRL of healthy men. The ability of postprandial TRL at 3 h (early postprandial period) and 5 h (late postprandial period) to affect cell viability and cycle in the THP-1 human monocytic cell line was also determined. In a randomized, crossover experiment, 14 healthy volunteers (Caucasian men) ate meals enriched (50 g/m<sup>2</sup> body surface area) in refined olive oil, high-palmitic sunflower oil, butter, and a mixture of vegetable and fish oils, which had ratios of oleic:palmitic acid (MUFA:SFA) of 6.83 (5.43), 2.36 (2.42), 0.82 (0.48), and 13.81 (7.08), respectively. The ratio of TG:CHOL in postprandial TRL was inversely correlated ( $r = -0.89$  to  $-0.99$ ) with the ratio of oleic:palmitic acid and with the MUFA:SFA ratio in the dietary fats ( $P < 0.05$ ). Postprandial TRL at 3 h preferentially increased the proportion of necrotic cells, whereas postprandial TRL at 5 h increased the proportion of apoptotic cells ( $P < 0.05$ ). Cell cycle analysis showed that postprandial TRL blocked the human monocytes in S-phase. Our findings suggest that the level of TG and CHOL into postprandial TRL is associated with the ratios of oleic:palmitic acid and MUFA:SFA in dietary fats, which determines the ability of postprandial TRL to induce cytotoxicity and disturb the cell cycle in THP-1 cells. J. Nutr. 137: 1999–2005, 2007.

## Introduction

The proatherogenic lipoprotein profile during postprandial metabolism of dietary fats is characterized by a transient rise in the circulating triacylglycerol (TG)<sup>9</sup> concentration (1,2). In the course of postprandial lipemia, the quantity, degree of saturation, and chain length of dietary fatty acids mediate hydrolysis and lipid exchange of nascent TG-rich lipoproteins

(TRL) with cholesterol (CHOL)-rich lipoproteins (LDL and HDL) (3–5). High intakes of long-chain (n-3) PUFA (LCPUFA) [20:5(n-3), eicosapentaenoic acid (EPA) and 22:6(n-3), docosahexaenoic acid (DHA)] tend to decrease postprandial lipemia (6). Generally, fats rich in monounsaturated fatty acids (MUFA) [e.g. oleic acid, 18:1(n-9)] have been found to cause pronounced postprandial lipemia, with a tendency for large TRL particles (7). Short- and medium-chain SFA do not lead to marked postprandial lipemia, because they are absorbed and transported via the hepatic portal vein. Fats rich in long-chain SFA (e.g. palmitic acid, 16:0), such as butter, have been shown to exacerbate postprandial lipemic responses (8). Current dietary recommendations for reducing cardiovascular disease risk (9,10) emphasize reducing the intake of SFA (mainly palmitic acid) by increasing dietary fatty acids from MUFA (mainly oleic acid). However, no studies to our knowledge have yet evaluated the effects of variable amounts of SFA and MUFA in the meals on postprandial TRL remodeling.

Undegraded postprandial TRL and their remnants remain in blood until they are taken up by hepatic receptors; they can also

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<sup>3</sup> Supplemental Tables 1 and 2 are available with the online posting of this paper at [jn.nutrition.org](http://jn.nutrition.org).

<sup>8</sup> S.L. and B.B. are considered equal first authors.

<sup>9</sup> Abbreviations used: cdk, cyclin-dependent kinase; CETP, cholesterol ester transfer protein; CHOL, cholesterol; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HPSO, high-palmitic sunflower oil; LCPUFA, long-chain (n-3) PUFA; LpL, lipoprotein lipase; netAUC, net incremental area under the curve; ROO, refined olive oil; TG, triacylglycerol; TRL, TG-rich lipoprotein; VEFO, mixture of vegetable and fish oil.

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interact with circulating cells, cross the endothelial layer, and become trapped within the subendothelial space (11). Additionally, recruitment of monocytes into atherogenic foci and their retention within the atherogenic lesion contribute to the progression of plaque development (12). In plaques, monocyte-derived cells (macrophages) recognize postprandial TRL and modified forms of LDL and produce secretory factors that attract and activate smooth muscle cells, other immune cells, and more monocytes (13). In advanced plaques, necrotic lipid-filled cores are formed after lipoprotein-induced cytotoxicity (14). Understanding how monocytes differentiate into macrophages to form foam cells *in vitro* and *in vivo* has been the focus of many studies (15,16). However, unprocessed monocytes represent a large pool of circulating precursors that coexist in the blood stream with lipoproteins, mainly postprandial TRL, that carry the majority of circulating TG after a high-fat meal and more CHOL molecules per particle than does LDL (17). Because clinical studies have shown an association between hyperlipidemia and elevated numbers of circulating monocyte-derived phosphatidylserine-positive microvesicles (18), we questioned whether postprandial metabolism of dietary fats might represent a critical element in the survival of monocytes, before they are differentiated. Indeed, phosphatidylserine externalized by apoptotic cells is thrombogenic (19) and if removal of apoptotic cells becomes compromised, secondary necrosis may ensue and propagate inflammation (20). This could be especially important, because monocytes act not only as versatile phagocytic cells under macrophage phenotype but also as a source of migratory dendritic cells able to activate antigen-specific T lymphocytes (21).

Consistent with a previous study suggesting a role for the ratios of oleic:palmitic acid and MUFA:SFA of natural fats in human postprandial events (22), we have herein extended our investigation to further explore the influence of such dietary determinant on the ratio of TG:CHOL in TRL of healthy men at early (3 h) and late (5 h) postprandial periods. In addition, whether postprandial TRL at 3 and 5 h promote changes in cell viability and cycling in THP-1 human monocytic cell line was also assessed.

## Subjects and Methods

**Subjects and study design.** Fourteen healthy, Caucasian male non-smokers [mean  $\pm$  SD: BMI,  $23.9 \pm 1.9$  kg/m<sup>2</sup>; 22–37 y (range):  $27 \pm 7$  y] participated in this study. The number of subjects provided the power to detect differences at  $P < 0.05$  and  $1 - \beta = 0.83$ . The mean fasting total, LDL, and HDL CHOL and plasma TG concentrations of the participants at screening were  $4.09 \pm 0.31$ ,  $2.34 \pm 0.85$ ,  $1.39 \pm 0.21$ , and  $0.86 \pm 0.27$  mmol/L, respectively. More details on subjects and study design have been recently reported (22). Briefly, this was a randomized crossover study that included a washout period of 1 wk of consuming a National Cholesterol Education Program step I diet and an additional 1-wk adaptation period between diets supplemented with refined olive oil (ROO), high-palmitic sunflower oil (HPSO), butter, or a mixture of vegetable and fish oils (VEFO). Immediately after the 12-h-blood samples from fasting were taken from a cubital vein catheterized with a small-bore extension set and a Smartsite needleless valve port, the subjects consumed a fat-rich meal consisting of the corresponding dietary fats (ROO, HPSO, butter, or VEFO, 50 g/m<sup>2</sup> body surface area). Subjects were asked to consume the meal within 15 min. Fatty acid composition of meals was determined according to the method described in EC/796/2002 (23) (Supplemental Table 1). Blood samples were drawn every hour for a total of 8 h into precooled tubes containing sodium citrate (final concentration, 0.129 mmol/L) to measure plasma lipids and to prepare postprandial TRL. All protocols were approved before the start of the study by the Human Clinical Commission and the Ethics Committee of Hospitales Universitarias Virgen del Rocío (SAS, Seville) and informed consent was obtained from each subject. The study conformed with the principles set out in the Helsinki Declaration.

**Human postprandial lipoprotein isolation.** The isolation of TRL with a Svedberg flotation unit  $>400$  from freshly postprandial plasma samples collected 3 and 5 h after consumption of the test meals was performed as described previously (24). These time points were chosen to compare the most recently (nascent) secreted particles by the intestine with remnant postprandial particles. Total TG and CHOL in postprandial TRL were measured using enzyme-based colorimetric kits supplied by Thermo Trace (Triglycerides GPO and Infinity Cholesterol). For fatty acid composition of postprandial TRL, we used a simple and rapid 1-step lipid extraction and fatty acid methyl esters procedure according to the method of Garces and Mancha (25) with some modifications (Supplemental Table 2). Postprandial TRL were then stored at  $-70^{\circ}\text{C}$  until cell studies were conducted.

**Cell culture.** Nonadherent cells of the human monocytic THP-1 cell line (TIB-202; American Type Culture Collection) were cultured in RPMI 1640 medium supplemented with L-glutamine, penicillin, streptomycin, and 10% heat-inactivated fetal bovine serum at  $37^{\circ}\text{C}$  in a humidified atmosphere of 5% CO<sub>2</sub> in air. For treatment, 300,000 cells/mL were incubated in medium containing 0.5% FBS for 24 h. The cells were then stimulated with postprandial TRL at a final concentration of 100  $\mu\text{g}$  TG/mL for an additional 24 or 48 h.

**Flow cytometric analysis.** Early events associated with apoptosis and necrosis were evaluated using the binding of Annexin V to detect the translocation of phosphatidylserine from the inner side to the outer leaflet of the plasma membrane on apoptotic cells and propidium iodide to DNA on necrotic cells, as described in Vybrant Apoptosis Assay kit number 3 (Molecular Probes). Analysis of stained cells was performed measuring the fluorescence emission on EPICS XL flow cytometer (Beckman Coulter) at 530 nm and 585 nm for fluorescein isothiocyanate and propidium iodide, respectively, using EXPO32 Software (Beckman Coulter). At least 10,000 cells were analyzed and gated according to light scatter properties.

Monocytes were treated as described (26) and analyzed for cell cycle distribution with an EPICS XL flow cytometer (Beckman Coulter) and EXPO32 software (Beckman Coulter). Red fluorescence (585 nm) was evaluated on a linear scale and pulse width analysis was used to exclude cell doublets and aggregates from the analysis. Cells with DNA content between 2N and 4N were designated as being in the G<sub>1</sub>/G<sub>0</sub>-, S-, or G<sub>2</sub>/M-phase of the cell cycle. The number of cells in each compartment of the cell cycle was expressed as a percentage of the total number of cells present.

**Statistical analysis.** Individual data from each subject were plotted and evaluated qualitatively. Statistical analyses were carried out to compare the effects of each fat on the fasting and postprandial values and to analyze the values from each fat at different time intervals. The net incremental area under the curve (netAUC), including the entire incremental area below the curve and the area below the fasting concentration, was analyzed by a 1-factor repeated-measures ANOVA. A Bonferroni correction or Dunnett's comparison was used for the post hoc detection of significant pairwise differences. After checking for interaction, outcome variables were tested with 2-way ANOVA. The netAUC was calculated by the trapezoidal method using Microsoft EXCEL 2000 v.9 (Microsoft). Univariate correlation analysis between variables was performed with Pearson's product-moment correlations. Each experiment with cells was performed simultaneously on paired samples derived from incubation with 3- and 5-h postprandial TRL from each fat-meal. Within an experiment, triplicate wells were used for each condition or treatment. Results were expressed as means  $\pm$  SD. Comparisons for the differences between means were performed using the Student's *t* test. The data were analyzed using STATVIEW v.5 for WINDOWS (SAS Institute). The designated level of significance was  $P < 0.05$ .

## Results

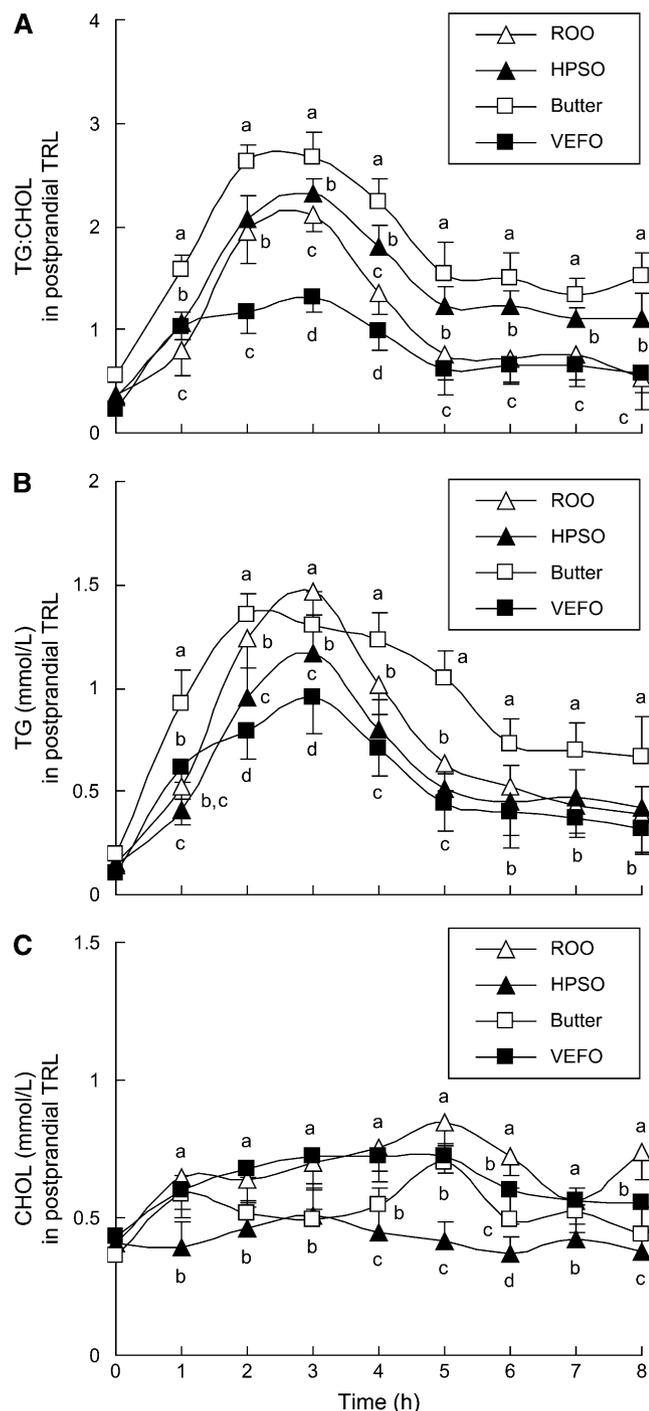
**TG and CHOL in postprandial TRL.** The ratios of TG:CHOL in postprandial TRL peaked at 2–3 h and fell thereafter. Values returned to baseline 5 h after the ROO and VEFO meals. They

remained significantly higher than baseline after the HPSO and butter meals (Fig. 1A). The TG:CHOL ratio was higher ( $P < 0.05$ ) at all times after the butter meal and lower ( $P < 0.05$ ) at 2–4 h after the VEFO meal than after the other meals. The postprandial TG:CHOL ratio at 0–2 h was similar after the ROO and HPSO meals, but differed ( $P < 0.05$ ) at 3–8 h. At 3 h postprandial, the ROO and VEFO meals resulted in higher and lower TG concentrations, respectively, compared with the other fat meals ( $P < 0.05$ ; Fig. 1B). These values were markedly

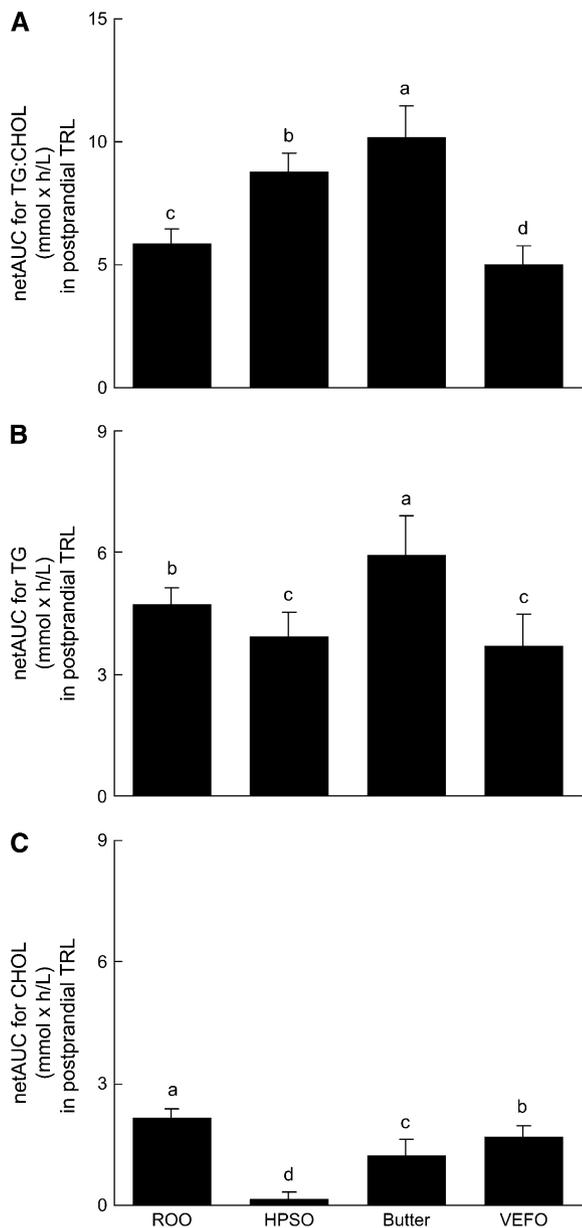
reduced at 5 h postprandial and differed ( $P < 0.05$ ) among butter, ROO, and HPSO or VEFO meals. The concentration of CHOL in postprandial TRL was higher ( $P < 0.05$ ) after ROO and VEFO meals at 3 h than after the other meals. At 5 h postprandial, TRL CHOL concentrations were greater ( $P < 0.05$ ) after the ROO meal followed by the VEFO or butter and HPSO meals (Fig. 1C). The postprandial response (netAUC) for TG:CHOL in TRL was lowest after the VEFO meal, followed by the ROO, HPSO, and butter meals, all of which differed from one another ( $P < 0.05$ ; Fig. 2A). The netAUC for TG were in the order of butter > ROO > HPSO = VEFO (Fig. 2B), whereas the order was ROO > VEFO > butter > HPSO for the netAUC for CHOL (all  $P < 0.05$ ; Fig. 2C). The slope of the curves for TG:CHOL, TG, and CHOL in postprandial TRL was also calculated between 3 and 5 h to represent the late phase of the postprandial period. Based on the generation of a first order polynomial equation (including both the intercept and slope), the ROO meal ( $y = 4.107 - 0.68x$ ,  $r = -0.99$ ) induced a greater rate of TG:CHOL clearance in postprandial TRL compared with the HPSO ( $y = 3.951 - 0.54x$ ,  $r = -0.99$ ), butter ( $y = 4.378 - 0.56x$ ,  $r = -0.98$ ), and VEFO ( $y = 2.393 - 0.36x$ ,  $r = -0.99$ ) meals. The order for the slopes of TG clearance curves was ROO > HPSO > VEFO > butter ( $P < 0.05$ ). However, the rate of CHOL accumulation in postprandial TRL was greater after the butter meal compared with the ROO meal ( $P < 0.05$ ), whereas the concentration of CHOL did not change after the VEFO meal and was apparently cleared after the HPSO meal. The netAUC for TG:CHOL was negatively correlated with the ratio of oleic:palmitic acid ( $r = -0.86$ ,  $P < 0.05$ ) and with the MUFA:SFA ratio ( $r = -0.99$ ,  $P < 0.05$ ) in postprandial TRL. The ratio of oleic:palmitic acid in the fat meals was negatively correlated with the TG:CHOL ratio in TRL at 3 and 5 h postprandial (Fig. 3A) and positively correlated with the ratio of oleic:palmitic acid in postprandial TRL at 3 h ( $r = 0.92$ ,  $P < 0.05$ ) and 5 h ( $r = 0.91$ ,  $P < 0.05$ ). The MUFA:SFA ratio in the fat meals was negatively correlated with the TG:CHOL ratio in TRL at 3 and 5 h postprandial (Fig. 3B) and positively correlated with the ratio of oleic:palmitic acid in postprandial TRL at 3 h ( $r = 0.97$ ,  $P < 0.05$ ) and 5 h ( $r = 0.96$ ,  $P < 0.05$ ).

**Effects of postprandial TRL on THP-1 cell viability.** Postprandial TRL (100 mg triglycerides/L) at 3 and 5 h after the different fat-enriched meals were cytotoxic for THP-1 cells (Table 1). This effect was nonlinear and dose-dependent from 0 to 100 mg/L; doses >100 mg/L did not enhance cytotoxicity (data not shown). Treatment of cells with TRL obtained at 3 h postprandially for 24 h decreased the proportion of viable cells and increased the proportion of necrotic cells (all  $P < 0.05$ ), but there were no different effects between meals. Necrotic cells increased with time of incubation (for 48 h), in particular with postprandial TRL at 3 h after the butter and VEFO meals ( $P < 0.05$ ). However, cells became mainly apoptotic with postprandial TRL at 5 h (all  $P < 0.05$ ). This effect with postprandial TRL at 5 h was higher after the VEFO meal when cells were treated for 24 h ( $P < 0.05$ ) and lower after the ROO meal when cells were treated for 48 h ( $P < 0.05$ ).

**Effects of postprandial TRL on THP-1 cell cycling.** Postprandial TRL at 3 and 5 h after the different fat-enriched meals affected the cell cycle distribution of THP-1 cells (Table 2). Treatment of cells for 24 and 48 h with TRL obtained at 3 h postprandially caused an accumulation of S-phase of cell cycle, whereas those cells in the G<sub>1</sub>/G<sub>0</sub>- and G<sub>2</sub>/M-phase decreased inversely over the same time of incubation (all  $P < 0.05$ ). The



**FIGURE 1** TG:CHOL (A) and concentrations of TG (B) and CHOL (C) in postprandial TRL of healthy men after the test meals enriched in ROO, HPSO, butter, and VEFO. Data are presented as means  $\pm$  SD,  $n = 14$ . Means at a time without a common letter differ,  $P < 0.05$ .

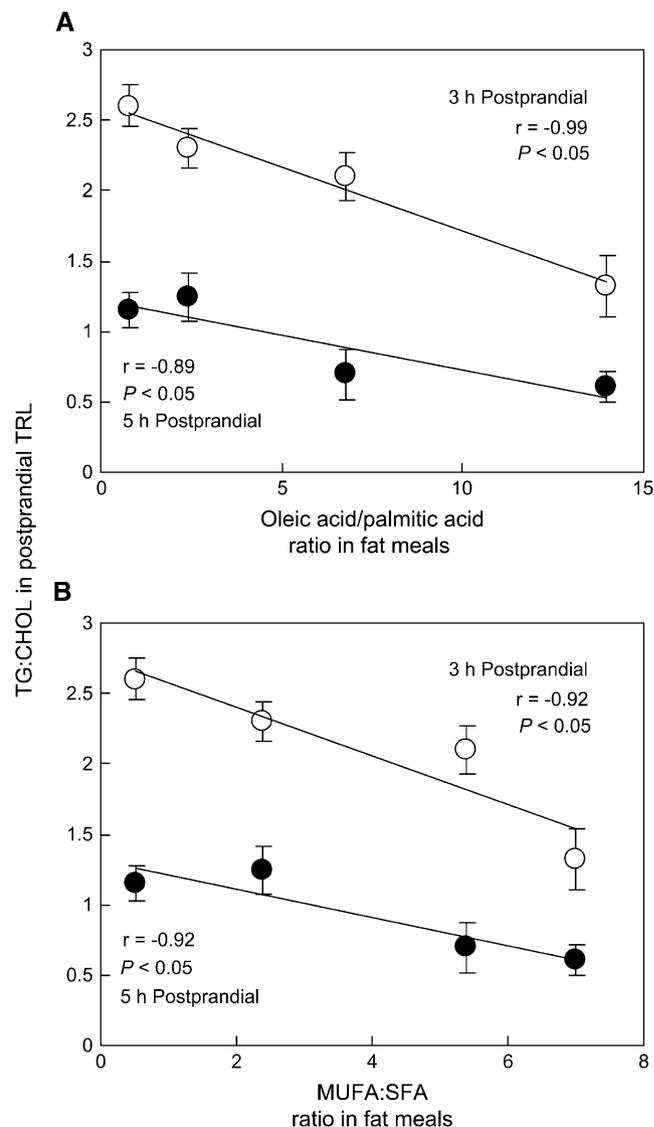


**FIGURE 2** NetAUC for TG:CHOL (A), TG (B), and CHOL (C) in postprandial TRL of healthy men after the test meals enriched in ROO, HPSO, butter, and VEFO. Data are presented as means  $\pm$  SD,  $n = 14$ . Bars without a common letter differ,  $P < 0.05$ .

percentage of S-phase cells was increased with postprandial TRL at 5 h (all  $P < 0.05$ ), which consistently resulted in a loss of cells in the  $G_1/G_0$ -phase and very few cells if any in the  $G_2/M$ -phase. There were no major differences between meals or incubation times.

## Discussion

Our findings provide evidence that the ratios of oleic:palmitic acid and MUFA:SFA in dietary fats modulate the ratio of TG:CHOL in postprandial TRL of healthy men. We found an inverse and significant correlation of the netAUC for TG:CHOL in postprandial TRL with the ratio of oleic:palmitic acid and with the MUFA:SFA ratio in dietary fats, which resulted in values of netAUC for TG:CHOL in the order of butter > HPSO > ROO > VEFO. The high-fat meals had no influence on fasting



**FIGURE 3** Correlations of TG:CHOL ratio in postprandial TRL at 3 h (A) and 5 h (B) of healthy men with the ratio of oleic:palmitic acid and with the MUFA:SFA ratio in ROO, HPSO, butter, and VEFO. Data are presented as means  $\pm$  SD,  $n = 14$ .

TG and CHOL, indicating that postprandial processing of TRL mostly contributed to changes in the TG:CHOL response. The mechanisms responsible for the aforementioned remodeling of postprandial TRL are not entirely understood but could be the result of different clearance rates for postprandial TRL to the type of dietary fat in the meal, affected by the extent of TG intraluminal lipolysis and ability of TG-depleted particles to accept CHOL from donors. Previous studies reported that dietary fatty acids might modulate the clearance of TG in postprandial TRL by lipoprotein lipase (LpL)-mediated lipolysis (3,5). Fatty acids have been shown to exert some regulatory cellular effects on LpL, but they also compete for LpL binding sites (27). Notably, TG molecules enriched in oleic acid, a MUFA, were found to be more efficiently hydrolyzed than those containing palmitic acid, an SFA (8), which supports the possibility that the ratios of oleic:palmitic acid and MUFA:SFA in dietary fats may modulate LpL function after the ingestion of high-fat meals. Indeed, the VEFO meal (containing 1.0% EPA and 2.3% DHA) induced the lowest netAUC for TG:CHOL

**TABLE 1** Analysis of cytotoxicity by flow cytometry of THP-1 human monocytic cell line treated for 24 and 48 h with 3- and 5-h postprandial TRL from different fats<sup>1</sup>

Cell condition and treatment	3-h Postprandial		5-h Postprandial	
	24 h	48 h	24 h	48 h
Alive	%			
Untreated	76.5 ± 1.8 <sup>a</sup>	75.4 ± 2.3 <sup>a</sup>	75.1 ± 1.1 <sup>a</sup>	74.8 ± 2.1 <sup>a</sup>
TRL-ROO	72.1 ± 1.5 <sup>b</sup>	63.1 ± 1.7 <sup>b**</sup>	68.3 ± 2.1 <sup>b*</sup>	50.0 ± 2.5 <sup>b**</sup> **
TRL-HPSO	72.2 ± 2.2 <sup>b</sup>	59.9 ± 1.8 <sup>c**</sup>	67.7 ± 2.3 <sup>b*</sup>	42.7 ± 1.3 <sup>c**</sup> **
TRL-butter	71.7 ± 1.1 <sup>b</sup>	52.6 ± 3.6 <sup>d**</sup>	68.5 ± 2.0 <sup>b*</sup>	43.4 ± 1.9 <sup>c**</sup> **
TRL-VEFO	73.2 ± 1.7 <sup>b</sup>	51.6 ± 1.9 <sup>d**</sup>	64.5 ± 1.6 <sup>b*</sup>	45.2 ± 2.8 <sup>c**</sup> **
Apoptotic				
Untreated	9.9 ± 1.4	10.1 ± 1.6 <sup>b</sup>	9.4 ± 1.3 <sup>c</sup>	9.0 ± 1.5 <sup>c</sup>
TRL-ROO	10.4 ± 1.9	15.9 ± 2.4 <sup>**</sup>	18.2 ± 1.5 <sup>b*</sup>	30.9 ± 0.3 <sup>b**</sup> **
TRL-HPSO	11.0 ± 1.5	17.0 ± 1.3 <sup>**</sup>	18.4 ± 1.8 <sup>b*</sup>	33.1 ± 1.3 <sup>**</sup> **
TRL-butter	10.8 ± 1.5	17.3 ± 2.0 <sup>**</sup>	17.6 ± 1.1 <sup>b*</sup>	33.6 ± 1.4 <sup>**</sup> **
TRL-VEFO	10.0 ± 1.4	17.7 ± 1.2 <sup>**</sup>	21.1 ± 1.5 <sup>a*</sup>	34.4 ± 1.3 <sup>**</sup> **
Necrotic				
Untreated	13.6 ± 1.1 <sup>b</sup>	14.5 ± 2.5 <sup>c</sup>	15.5 ± 1.2	16.2 ± 1.0 <sup>a</sup>
TRL-ROO	17.5 ± 1.1 <sup>a</sup>	21.0 ± 2.2 <sup>b**</sup>	13.5 ± 2.6 <sup>*</sup>	19.1 ± 1.3 <sup>b**</sup> **
TRL-HPSO	16.8 ± 1.7 <sup>a</sup>	23.1 ± 1.7 <sup>b**</sup>	13.9 ± 1.5 <sup>*</sup>	24.2 ± 1.2 <sup>c**</sup> **
TRL-butter	17.5 ± 1.5 <sup>a</sup>	30.1 ± 4.1 <sup>**</sup>	13.9 ± 1.9 <sup>*</sup>	23.0 ± 0.5 <sup>c**</sup> **
TRL-VEFO	16.8 ± 1.2 <sup>a</sup>	30.7 ± 1.7 <sup>**</sup>	14.4 ± 1.2 <sup>*</sup>	20.4 ± 1.5 <sup>b**</sup> **

<sup>1</sup> Values are means ± SD, *n* = 4. For each cell condition, means in a column with superscripts without a common letter differ, *P* < 0.05. \*, \*\*Different from 3-h postprandial TRL or 24 h of incubation time, respectively, *P* < 0.05.

in postprandial TRL, in agreement with studies suggesting that (n-3) LCPUFA may further enhance LpL activity (28) and binding affinity to TRL (29) in the fed state. These observations, and our data on fatty acid composition in TRL at 3 and 5 h showing selective clearance of oleic acid (MUFA), reinforce the

**TABLE 2** Cell cycle analysis by flow cytometry of THP-1 human monocytic cell line treated for 24 and 48 h with 3- and 5-h postprandial TRL from different fats<sup>1</sup>

Cell stage and treatment	3-h Postprandial		5-h Postprandial	
	24 h	48 h	24 h	48 h
G <sub>1</sub> /G <sub>0</sub>	%			
Untreated	75.9 ± 1.1 <sup>a</sup>	74.8 ± 1.3 <sup>a</sup>	74.4 ± 1.6 <sup>a</sup>	74.1 ± 2.1 <sup>a</sup>
TRL-ROO	73.2 ± 1.2 <sup>b</sup>	71.5 ± 1.8 <sup>b</sup>	69.8 ± 1.1 <sup>b*</sup>	62.6 ± 2.3 <sup>b**</sup> **
TRL-HPSO	71.6 ± 1.3 <sup>b</sup>	70.2 ± 2.0 <sup>b</sup>	70.8 ± 1.8 <sup>b</sup>	61.3 ± 3.3 <sup>b**</sup> **
TRL-butter	73.4 ± 1.2 <sup>b</sup>	72.0 ± 1.7 <sup>b</sup>	70.2 ± 1.3 <sup>b*</sup>	60.8 ± 2.6 <sup>b**</sup> **
TRL-VEFO	73.6 ± 1.4 <sup>b</sup>	72.2 ± 1.6 <sup>b</sup>	67.8 ± 2.0 <sup>b*</sup>	60.3 ± 1.8 <sup>b**</sup> **
S				
Untreated	18.3 ± 1.6 <sup>b</sup>	19.0 ± 1.9 <sup>b</sup>	20.1 ± 1.8 <sup>b</sup>	20.3 ± 1.9 <sup>b</sup>
TRL-ROO	22.2 ± 1.7 <sup>a</sup>	23.9 ± 1.0 <sup>a</sup>	30.0 ± 1.2 <sup>a*</sup>	37.3 ± 2.2 <sup>a**</sup> **
TRL-HPSO	24.5 ± 1.0 <sup>a</sup>	26.5 ± 2.1 <sup>a</sup>	28.7 ± 1.4 <sup>a*</sup>	38.6 ± 2.4 <sup>a**</sup> **
TRL-butter	23.2 ± 1.6 <sup>a</sup>	25.6 ± 1.1 <sup>a</sup>	29.3 ± 1.5 <sup>a*</sup>	38.8 ± 2.1 <sup>a**</sup> **
TRL-VEFO	23.0 ± 1.6 <sup>a</sup>	25.3 ± 1.4 <sup>a</sup>	32.1 ± 1.3 <sup>a*</sup>	39.6 ± 1.9 <sup>a**</sup> **
G <sub>2</sub> /M				
Untreated	5.8 ± 0.1 <sup>a</sup>	6.2 ± 0.5 <sup>a</sup>	5.5 ± 0.6 <sup>a</sup>	5.6 ± 0.5 <sup>a</sup>
TRL-ROO	4.6 ± 0.5 <sup>b</sup>	4.5 ± 0.8 <sup>b</sup>	0.2 ± 0.1 <sup>b*</sup>	0.1 ± 0.0 <sup>b*</sup>
TRL-HPSO	3.9 ± 0.2 <sup>c</sup>	3.3 ± 0.5 <sup>b</sup>	0.5 ± 0.4 <sup>b*</sup>	0.1 ± 0.0 <sup>b*</sup>
TRL-butter	3.4 ± 0.5 <sup>c</sup>	2.4 ± 0.4 <sup>c**</sup>	0.5 ± 0.4 <sup>b*</sup>	0.4 ± 0.3 <sup>b*</sup>
TRL-VEFO	3.4 ± 0.6 <sup>c</sup>	2.5 ± 0.3 <sup>c**</sup>	0.1 ± 0.0 <sup>b*</sup>	0.1 ± 0.0 <sup>b*</sup>

<sup>1</sup> Values are means ± SD, *n* = 4. For each cell condition, means in a column with superscripts without a common letter differ, *P* < 0.05. \*, \*\*Different from 3-h postprandial TRL or 24 h of incubation time, respectively, *P* < 0.05.

view of dietary fatty acid-mediated effects on TG lipolysis and TRL uptake postprandially that would explain in part the temporal patterns in TG concentration and TG:CHOL of postprandial TRL to high-fat meals with different ratios of oleic:palmitic acid and MUFA:SFA.

In addition, delayed postprandial clearance of TG has been reported to increase the exchange of lipids between postprandial TRL and CHOL-rich lipoproteins mediated by CHOL ester transfer protein (CETP) (4). Such reciprocal exchange leads to the formation of postprandial TRL enriched in CHOL, whereas CHOL-rich lipoproteins are enriched in TG (30). The present study shows that the slopes for TG:CHOL and TG clearance in postprandial TRL were the steepest after the ROO meal. We also found evidence of CHOL enrichment of postprandial TRL by measuring the netAUC for CHOL after the VEFO, ROO, HPSO, and butter meals. The ROO meal induced the largest netAUC for CHOL in postprandial TRL, suggesting a high lipid exchange turnover with CHOL-rich lipoproteins. However, oleic acid is a poor activator of CETP when compared with palmitic acid (31). As proposed in previous trials, the transferability of CHOL from donors might relate to its affinity for CETP, which varies according to the fatty acid composition of the diet (5), but also to the (surface) size of acceptors (32). Accordingly, the ingestion of ROO-containing meals has been shown to cause the formation of large postprandial TRL (7), which may thereby facilitate their lipid transfer with CHOL-rich lipoproteins (33). A lower netAUC for CHOL in postprandial TRL after the VEFO meal was consistent with the known CETP-lowering effect of (n-3) LCPUFA (34). We also observed that the butter meal induced the largest netAUC for TG in postprandial TRL and a positive change of CHOL in postprandial TRL from 3 to 5 h, suggesting that a delay in postprandial TRL clearance contributed to their CHOL enrichment in the late postprandial period. This is in agreement with the postprandial response pattern of CETP activity to palmitic acid-enriched meals (5).

We noticed that postprandial TRL were cytotoxic for the THP-1 human monocytic cell line. Postprandial TRL at 3 h preferentially increased necrotic cells, whereas postprandial TRL at 5 h resulted in a shift to increase the apoptotic cells. Unlike apoptosis, necrosis is characterized by cell swelling and bursting before release of cell content, which may induce an inflammatory response. The necrotic effect of postprandial TRL at 3 h was in the following order: butter = VEFO > HPSO > ROO, whereas the order of necrotic and apoptotic effects with postprandial TRL at 5 h was: butter = HPSO > VEFO = ROO. Therefore, postprandial TRL from ROO were less cytotoxic for monocytes and probably more beneficial by preventing acute inflammatory response from necrotic monocytes than VEFO, HPSO, and butter. A number of studies have shown that SFA were quickly incorporated into phospholipids and generate specific pro-cytotoxic lipid species or signaling molecules that were not produced in response to MUFA (35–37). Likewise, oleic acid was found to play an important role in rescuing cells from palmitic acid-induced cytotoxicity in a variety of systems (38). Cytotoxicity of postprandial TRL after the VEFO meal confirmed previous *in vitro* and *in vivo* studies showing the capability of EPA and DHA to inhibit cell survival by affecting multiple pathways (39). Our study supports the notion that TG in postprandial TRL may evoke procoagulant (18,19) and proinflammatory (20,40) changes by inducing apoptosis and necrosis of circulating monocytes. However, CHOL or other lipid components of TRL might also be responsible for cytotoxicity toward monocytes (41–43). We do not exclude the possibility that other minor fatty acids could also be involved. Further

studies are required to determine whether cytotoxicity of postprandial TRL on THP-1 cells are due to fatty acids intracellularly or extracellularly produced by the action of endogenous lipases (44), which could explain the progressive effects from 24 to 48 h of incubation.

Cell cycle analysis showed that postprandial TRL blocked the THP-1 human monocytic cell line in S-phase and diminished the proportion in G<sub>2</sub>/M-phase, reflecting inhibition of S/G<sub>2</sub> transit or S-phase cell cycle arrest. In addition, the proportion of cells in S-phase increased with the incubation time (mainly with TRL at 5 h postprandial). The decondensation of chromatin in this phase is favored by a decrease of sphingomyelin and an increase of the CHOL free fraction (not linked to sphingomyelin) in the chromatin CHOL, which stimulates the activities of cyclin-dependent kinases (cdk) and contributes to kinase activation and G<sub>1</sub>- to S-phase progression (45). Members of the cdk-inhibitor family (including p21<sup>waf1</sup>, p27<sup>kip1</sup>, and p57<sup>kip2</sup>) promote cell-cycle arrest by binding and inhibiting cyclin E-cdk2 and the D cyclin-cdk4/6 protein complexes in response to toxic stress (46). Importantly, fatty acids and other PPAR $\gamma$  ligands may modify the expression and/or protein degradation at least of p21<sup>waf1</sup> and p27<sup>kip1</sup> (47–49), supporting a model wherein fatty acids from TG of postprandial TRL lead to regulation of the cell cycle.

In conclusion, data presented here show a link between the ratios of oleic:palmitic acid and MUFA:SFA in dietary fats and the level of TG and CHOL in postprandial TRL of healthy men. Our observations further support the importance of the magnitude and duration of the postprandial response to high-fat meals in postprandial TRL remodeling. In agreement with a general belief that MUFA are less atherogenic than SFA, postprandial TRL from ROO elicited less adverse cytotoxic and cell cycle effects on THP-1 cells than dietary fats rich in SFA (butter and HPSO) or containing a low amount of (n-3) LCPUFA (VEFO). This study could be of particular relevance for the clinical outcomes of subjects with visceral obesity, insulin resistance, type 2 diabetes, type III hyperlipidemia, familial combined hyperlipidemia, and other constitutional, environmental, hormonal, and genetic factors who have pathologically exacerbated and delayed postprandial response to the ingestion of dietary fats.

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