

## RESEARCH ARTICLE

# The effects of exogenous fatty acids and niacin on human monocyte-macrophage plasticity

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**Scope:** Macrophage plasticity allows adapting to different environments, having a dual activity in inflammatory-related diseases. Our hypothesis is that the type of dietary fatty acids into human postprandial triglyceride-rich lipoproteins (TRLs), alone or in combination with niacin (vitamin B3), could modulate the plasticity of monocytes-macrophages.

**Methods and results:** We isolated TRLs at the postprandial peak from blood samples of healthy volunteers after the ingestion of a meal rich in saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) or MUFAs plus omega-3 long-chain polyunsaturated fatty acids (LCPUFAs). Autologous monocytes isolated at fasting were first induced to differentiate into naïve macrophages. We observed that postprandial TRL-MUFAs, particularly in combination with niacin, enhance competence to monocytes to differentiate and polarise into M2 macrophages. Postprandial TRL-SFAs made polarised macrophages prone to an M1 phenotype. In contrast to dietary SFAs, dietary MUFAs in the meals plus immediate-release niacin primed circulating monocytes for a reduced postprandial pro-inflammatory profile.

**Conclusion:** Our study underlines a role of postprandial TRLs as a metabolic entity in regulating the plasticity of the monocyte-macrophage lineage and also brings an understanding of the mechanisms by which dietary fatty acids are environmental factors fostering the innate immune responsiveness in humans.

**Keywords:**

Fatty acids / Macrophages / Monocytes / Niacin / Postprandial state



Additional supporting information may be found in the online version of this article at the publisher's web-site

## 1 Introduction

Vitamin B3 (niacin or nicotinic acid) is a water-soluble vitamin that can be effective for treatment of chronic high-grade inflammatory diseases such as atherosclerosis [1]. Many of

beneficial effects of niacin are mediated via the G protein-coupled receptor 109A/hydroxycarboxylic acid 2 receptor (GPR109A/HCA2), which is highly expressed in adipose tissue and macrophages [2].

Serving to defend the organism from infection, damaged or activated cells, macrophages are a component of innate immunity with remarkable versatility of characteristic features that enables them to promote inflammation and to turn the inflammatory response off when it is no longer needed [3]. This macrophage phenotypic heterogeneity often depends on the microenvironment, particularly of the surrounding gradients of cytokines and growth factors, such as granulocyte-macrophage (GM-CSF) and macrophage

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**Abbreviations:** **DHA**, docosahexaenoic acid; **EPA**, eicosapentaenoic acid; **FA**, fatty acid; **GM-CSF**, granulocyte-macrophage colony-stimulating factor; **HPRT**, hypoxanthine phosphoribosyltransferase; **LCPUFA**, long-chain polyunsaturated fatty acid; **LPS**, lipopolysaccharide; **M-CSF**, macrophage colony-stimulating factor; **MUFA**, monounsaturated fatty acid; **RPLPO**, Ribosomal protein large P0; **SFA**, saturated fatty acid; **TRL**, triglyceride-rich lipoprotein

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(M-CSF) colony-stimulating factors, which are implicated in the differentiation of naïve macrophages from monocytes [4]. Another level of heterogeneity between macrophages is related to their activation/polarization status [5]. As one extreme of different states of macrophage activation, classically activated macrophages (also called M1 macrophages) are stimulated by microbial products such as Toll-like receptor (TLR) ligands such as lipopolysaccharide (LPS) or pro-inflammatory cytokines such as interferon  $\gamma$  (IFN $\gamma$ ) [6]. These M1 macrophages are characterized by a high expression of pro-inflammatory cytokines (e.g., IL-1 $\beta$ , TNF- $\alpha$ , and nicotinamide phosphoribosyltransferase, NAMPT), chemokines (e.g., CXCL10), chemokine receptors (e.g., CCR7), and costimulatory proteins (e.g., CD80 and TLR4). In contrast, alternatively activated macrophages (also called M2 macrophages) are stimulated by IL-4 or IL-13 and their typical characteristics include high levels of scavenging molecules, high expression of mannose (e.g., CD206) and galactose receptors, CD200R, transforming growth factor  $\beta$  (TGF- $\beta$ ), peroxisome proliferator-activated receptor  $\gamma$  (PPAR- $\gamma$ ), high levels of anti-inflammatory cytokines (e.g., IL-10), and low levels of pro-inflammatory cytokines [7].

In previous studies, the ingestion of fatty meals has been noticed to induce not only hypertriglyceridemia by virtue of an abrupt intestinal production of triglyceride-rich lipoproteins (TRLs) but also an increase in the plasma levels of cytokines and chemokines that prime the activation of circulating monocytes in humans [8–10]. It has been demonstrated that both monocytes and macrophages are functionally equipped with apoB48 receptor to take up postprandial TRLs, resulting in foam cell formation, which is the first cellular hallmark of the atherosclerotic process [11]. Interestingly, the severity of atherosclerosis is largely influenced by increased leukocyte activation and by long-lasting high circulating postprandial TRLs [12, 13]. The fatty acid composition in triglycerides of postprandial TRLs faithfully represents the fatty acid composition of dietary fats ingested. Therefore, the postprandial TRLs constitute an exceptional physiological metabolic entity to explore the relevance of dietary, exogenous fatty acids in the human monocyte-macrophage biology. We previously demonstrated that postprandial TRLs regulate human macrophage-derived foam cells in a fatty acid-dependent manner (SFAs > MUFAs) [11, 14, 15]. To gain insights into these questions, the present study was aimed to assess whether the type of predominant fatty acids in postprandial TRLs, in particular those enriched in SFAs, MUFAs, or MUFAs plus omega-3 long-chain PUFAs (LCPUFAs), alone or in combination with niacin is instrumental in the modulation of human monocyte-macrophage plasticity.

## 2 Materials and methods

This study was conducted according to Good Clinical Practice Guidelines and in line with the principles outlined in the Helsinki Declaration of the World Medical Association. In-

formed consent for the study was obtained. This study is part of a clinical trial registered at ClinicalTrials.gov (identifier: NCT02061267).

### 2.1 TRL isolation, quality, and triglyceride analysis

Oral fat emulsions were prepared according to the method described by the Patent WO/2014/191597 [16] with water, sucrose (30 g/m<sup>2</sup> of body surface area), fat (50 g/m<sup>2</sup> of body surface area), emulsifier, and flavoring. Six male volunteers, aged 25–35 years, non-smokers, with no medical history of disease known, abnormality of haematological or biochemical parameters were recruited. After an overnight fasting period of 12 h, all of them were given, over three different occasions, an oral fat emulsion containing cow's milk cream (meal rich in SFAs), refined olive oil (meal rich in MUFAs) or refined olive plus a dose of omega-3 LCPUFAs, which consisted of 920 mg of eicosapentaenoic acid (EPA) and 760 mg of docosahexaenoic acid (DHA) (meal rich in MUFAs+omega-3 LCPUFAs). At the postprandial hypertriglyceridemic peak, i.e. 2–3 h following the ingestion of the oral fat emulsion, venous blood was collected into K<sub>3</sub>EDTA-containing Vacutainer tubes (BD). TRLs were isolated, pooled, and dialysed against cold PBS [17]. TRLs were then immediately stored at –80°C. The details of quality and triglyceride analysis of TRLs can be found in the Supporting Information.

### 2.2 Fat and TRL fatty acid composition

The fatty acid composition of cow's milk cream, refined olive oil, and refined olive oil plus omega-3 LCPUFAs was determined, in triplicate from the same lot, by the method described in EEC/796/2002 [18] using a gas chromatography system (Supporting Information Table 1). A similar procedure was followed for fatty acid composition in postprandial TRLs (named TRL-SFAs from cow's milk cream, TRL-MUFAs from refined olive oil, and TRL-PUFAs from refined olive oil plus omega-3 LCPUFAs) (Supporting Information Table 2). The detailed methods can be found in the Supporting Information.

### 2.3 Human monocyte isolation

The same six volunteers who took part as donors of postprandial TRLs participated as donors of monocytes. After an overnight fasting period of 12 h, peripheral blood samples were drawn from a large antecubital vein and collected into K<sub>3</sub>EDTA-containing tubes (BD). Peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation over a Ficoll-Histopaque (Sigma) gradient. Monocytes were isolated from PBMCs using CD14 microbeads and LS columns on a midiMACS system (Miltenyi Biotec). Monocytes were tested for purity by CD14 fluorescein isothiocyanate (FITC) labelling and fluorescence-activated cell sorter (FACS) analysis. The detailed method can be found in the Supporting Information.

## 2.4 Monocyte differentiation and polarization

Freshly monocytes were induced to differentiate into mature macrophages (M0) by using GM-CSF or M-CSF. M0 macrophages were induced to polarise into M1 or M2 macrophage subtype by long-established cocktail method. GM-M1 and GM-M2 macrophages are referred as those from GM-CSF-derived M0 macrophages. M-M1 and M-M2 macrophages are referred as those from M-CSF-derived M0 macrophages. The detailed methods can be found in the Supporting Information. All of these incubations were done in the absence or presence of autologous postprandial TRLs (100 µg triglycerides/mL) and niacin (100 nM). The experiments were conducted with only once thawed postprandial TRL samples.

## 2.5 MTT assay

Cell survival was assessed by the MTT colorimetric assay in all of experimental conditions of monocyte differentiation into macrophages and further polarization in which postprandial TRLs and/or niacin were added. This assay was based on the reduction of dimethylthiazolyldiphenyl-tetrazolium bromide to formazan crystals and was performed as described previously [19].

## 2.6 RNA extraction and qPCR

Total RNA was extracted from cells using TRIsure™ reagent (Bioline). RNA concentration was assessed in a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific). Reverse transcription was performed using 500 ng RNA and iScript™ cDNA Synthesis Kit (Bio-Rad). An amount of 20 ng of the resulting cDNA was used as a template for real-time PCR amplifications. The mRNA levels for specific genes were determined in a CFX96 system (Bio-Rad). Ribosomal protein large P0 (*Rplp0*) and hypoxanthine phosphoribosyltransferase (*Hprt*) were used as housekeeping genes. The sequences of the primers used in this study are shown in Supporting Information Table 3. Reactions were performed in triplicate and the change in mRNA expression was calculated by using the  $2^{-(\Delta\Delta C_t)}$  method. All data were normalised to endogenous reference (*Rplp0* and *Hprt*) gene content and expressed as relative to the control.

## 2.7 Enzyme-linked immunosorbent assay

Cytokine IL-1β, TNF-α, IL-10, and chemokine CXCL10 levels were measured in cell culture supernatants by ELISA (Dialone). Absorbance was read at 450 nm using a Multiskan Spectrum microplate reader (Thermo Labsystems). The quantification was made on the basis of calibration curves prepared for the cytokines and chemokine analyzed. Results are expressed as pg/mL of cell culture supernatant.

## 2.8 Human intervention: acute response to dietary fats and niacin

The same six volunteers who took part as donors of postprandial TRLs or fasting leukocytes participated in this part of the study. After an overnight fasting period of 12 h, all of them were given, over three different occasions, an oral fat emulsion (meal rich in SFAs, meal rich in MUFAs or meal rich in MUFAs plus omega-3 LCPUFAs) as indicated above and a single dose of 2 g immediate-release niacin (Twinlab, UT, USA). The participants also consumed the same test meal without fat (including niacin), as a control meal. Peripheral blood samples were drawn from a large antecubital vein at fasting and at the postprandial hypertriglyceridemic peak, i.e. 2–3 h, and collected into K<sub>3</sub>EDTA-containing tubes (BD). Monocytes were then isolated and differentiated into naïve GM-M0 and M-M0 macrophages as described above.

## 2.9 Statistical analysis

The statistics was performed using the GaphPad Prism v5.0. The experimental results are expressed as the mean ± SD of six independent experiments (one per volunteer) performed in triplicate. Statistical significances were assessed using a one-way analysis of the variance (ANOVA) for multiple comparisons with Tukey's *post hoc* test. For differences between postprandial TRL treatments in combination or not with niacin, we used a two-way ANOVA with Bonferroni's *post hoc* test. A *p*-value of less than 0.05 was considered statistically significant.

# 3 Results

## 3.1 GM-CSF and M-CSF pre-differentiation of human monocytes favors an M1 and M2 activation status, respectively, in polarized macrophages

In the context of the ability of GM-CSF and M-CSF to induce human monocyte differentiation into naïve M0 macrophages, we first compared their influence on the gene expression pattern of M1 and M2 markers in polarized macrophages (GM-M1, M-M1, GM-M2, and M-M2). As expected, the mRNA levels of M1 macrophage gene markers (*Cd80*, *Ccr7*, *Il1b*, *Tnfa*, and *Nampt*) were overrepresented in GM-M1 and M-M1 macrophages with respect to GM-M2 and M-M2 macrophages (Supporting Information Fig. 1). The mRNA levels of M2 macrophage gene markers (*Cd200r*, *Cd206*, *Il10*, *Tgfb*, and *Pparg*) were also overrepresented in GM-M2 and M-M2 macrophages with respect to GM-M1 and M-M1 macrophages. However, GM-M1 macrophages had enhanced transcriptional activity of M1 macrophage gene markers (*Cd80*, *Ccr7*, *Il1b*, and

*Tnfa*) when compared to M-M1 macrophages, whereas M-M2 macrophages had upregulated *Tgfb* and *Pparg* gene expression when compared to GM-M2 macrophages. Therefore, GM-M1 and M-M2 macrophages and their corresponding naïve GM-M0 and M-M0 macrophages were used for further evaluation of postprandial TRLs and niacin on human monocyte-macrophage plasticity.

### 3.2 GM-CSF but not M-CSF pre-differentiation of human monocytes increases mitochondrial activity in polarized macrophages. Role of postprandial TRLs and niacin

The mitochondrial activity by using the MTT assay was assessed in the above-mentioned cells. As shown in Fig. 1A, GM-M1 macrophages exhibited stimulated mitochondrial activity, remaining comparable the values of GM-M0, M-M0, and M-M2 macrophages. Autologous postprandial TRLs further increased, with no differences among TRL-SFAs, TRL-MUFAs, and TRL-PUFAs, the mitochondrial activity of GM-M1 macrophages (Fig. 1B) in a dose-dependent manner with a typical sigmoidal shape (Fig. 1C); however, no effects were observed in GM-M0 (Fig. 1B), M-M0 (Fig. 1D) or M-M2 (Fig. 1D and 1E) macrophages. Niacin did not affect the mitochondrial activity of any macrophage subtype (Fig. 1F).

### 3.3 Postprandial TRL-SFAs favors an M1 whereas niacin an M2 activation status in polarized macrophages

In gaining deeper insight into the role of autologous postprandial TRLs and niacin on human monocyte differentiation and macrophage polarization under the influence of GM-CSF and M-CSF, we observed that postprandial TRL-SFAs particularly promoted the transcriptional activity of M1 macrophage gene markers (*Cd80* and *Ccr7*) in GM-M1 macrophages (Fig. 2A and 2B). In contrast, niacin not only reduced the mRNA levels of *Cd80* and *Ccr7* genes in GM-M1 macrophages but also promoted the transcriptional activity of M2 macrophage gene marker *Cd206*, with no effect on *Cd200r* gene expression, in M-M2 macrophages (Fig. 2C and 2D). Postprandial TRLs did not affect the activation status of GM-M0 or M-M0 macrophages. Interestingly, when postprandial TRL-SFAs, TRL-MUFAs or TRL-PUFAs and niacin were added together, niacin abruptly reduced the postprandial-TRL-induced transcriptional activity of M1 macrophage gene markers in GM-M1 macrophages; in contrast, niacin increased the transcriptional activity of *Cd206* gene in M-M0 macrophages in the presence of any postprandial TRL and in M-M2 macrophages only in the presence of postprandial TRL-MUFAs or TRL-PUFAs. Similar findings were observed for the other M1 (*Il1b*, *Tnfa*, and *Nampt*) and M2 (*Il10*, *Tgfb*, and *Pparg*) gene markers (data not shown).

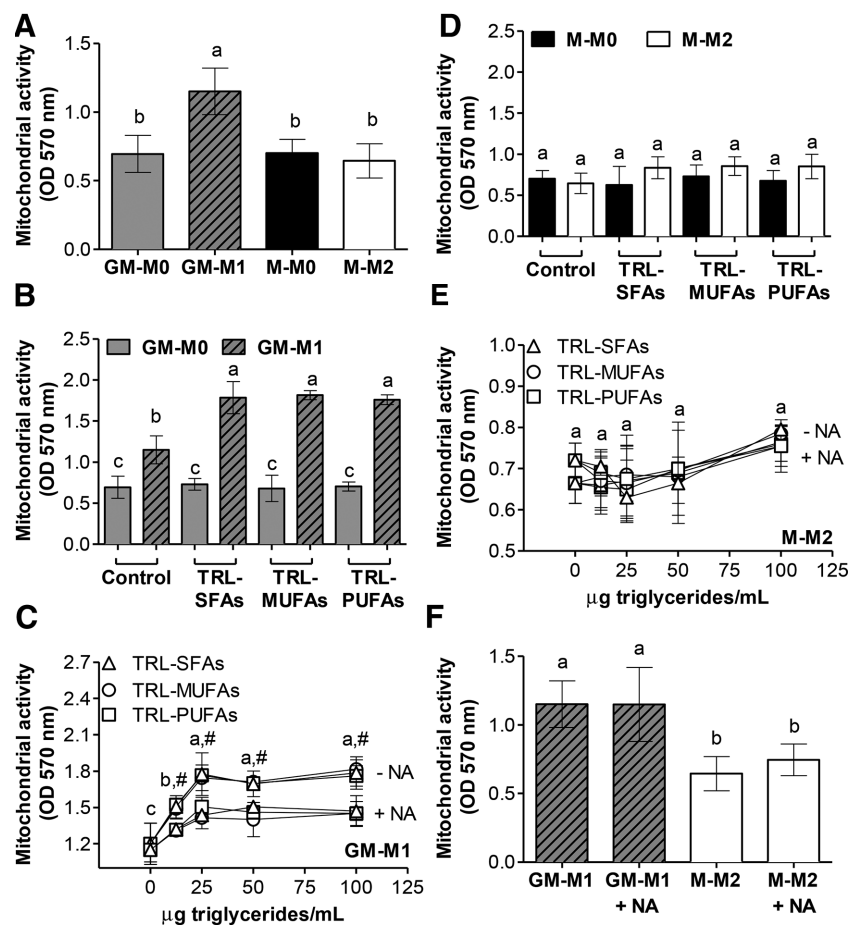
Furthermore, the release of pro-inflammatory cytokines IL-1 $\beta$  (Supporting Information Fig. 2A) and TNF- $\alpha$  (Supporting Information Fig. 2B) was increased into the medium of GM-M1 and GM-M0 macrophages only in the presence of postprandial TRL-SFAs. The release of pro-inflammatory chemokine CXCL10 was also increased into the medium of GM-M0 macrophages only in the presence of postprandial TRL-SFAs and into the medium of GM-M1 macrophages in the presence of any postprandial TRL but notably of postprandial TRL-SFAs (Supporting Information Fig. 2C). The release of anti-inflammatory cytokine IL-10 was increased into the medium of M-M0 macrophages only in the presence of postprandial TRL-PUFAs and decreased into the medium of M-M2 macrophages in the presence of any postprandial TRL (Supporting Information Fig. 2D). The majority of these effects on release of pro- and anti-inflammatory mediators were enhanced when postprandial TRLs and niacin were added together. In M-M2 macrophages, the release of IL-10 was decreased only in the presence of postprandial TRL-SFAs and niacin.

### 3.4 In contrast to a meal rich in SFAs, meals rich in MUFAs co-ingested with immediate-release niacin favors the priming of postprandial monocytes to naïve GM-M0 and M-M0 macrophages distant from an M1-like phenotype

In establishing the physiological relevance of these findings, naïve GM-M0 and M-M0 macrophages obtained from postprandial monocytes of healthy volunteers subjected to the above-mentioned oral fat emulsions and to a non-fat (control) meal, in combination with a single-dose of immediate-release niacin, were analyzed for gene expression of M1 and M2 markers, respectively. Co-ingestion of the SFA meal, but not of MUFA or PUFA meal, and niacin primed naïve GM-M0 macrophages by inducing the upregulation of M1 macrophage gene markers such as *Cd80* (Fig. 3A), *CCR7* (Fig. 3B), *Tlr4* (Fig. 3C), *Tnfa* (Fig. 3D), *Cxcl10* (Fig. 3E), and *Nampt* (Fig. 3F). The SFA meal also induced the downregulation of M2 macrophage gene markers such as *Cd200r* (Fig. 4A), *Cd206* (Fig. 4B), *Tgfb* (Fig. 4C), and *Pparg* (Fig. 4D) in M-M0 macrophages. In contrast, the co-ingestion of the MUFA or PUFA meal and niacin promoted the downregulation of *Nampt* gene in GM-M0 macrophages and the upregulation of *Cd200r* gene in M-M0 macrophages when compared to the control meal and niacin. Furthermore, the MUFA and PUFA meals reduced the transcriptional activity of *Cd206* and *Tgfb* genes but to a lesser extent than the SFA meal and niacin.

## 4 Discussion

Systemic dyslipidaemias are involved in the etiology of type-2 diabetes and metabolic syndrome promoting atherosclerosis. The accumulation of monocyte-derived macrophages



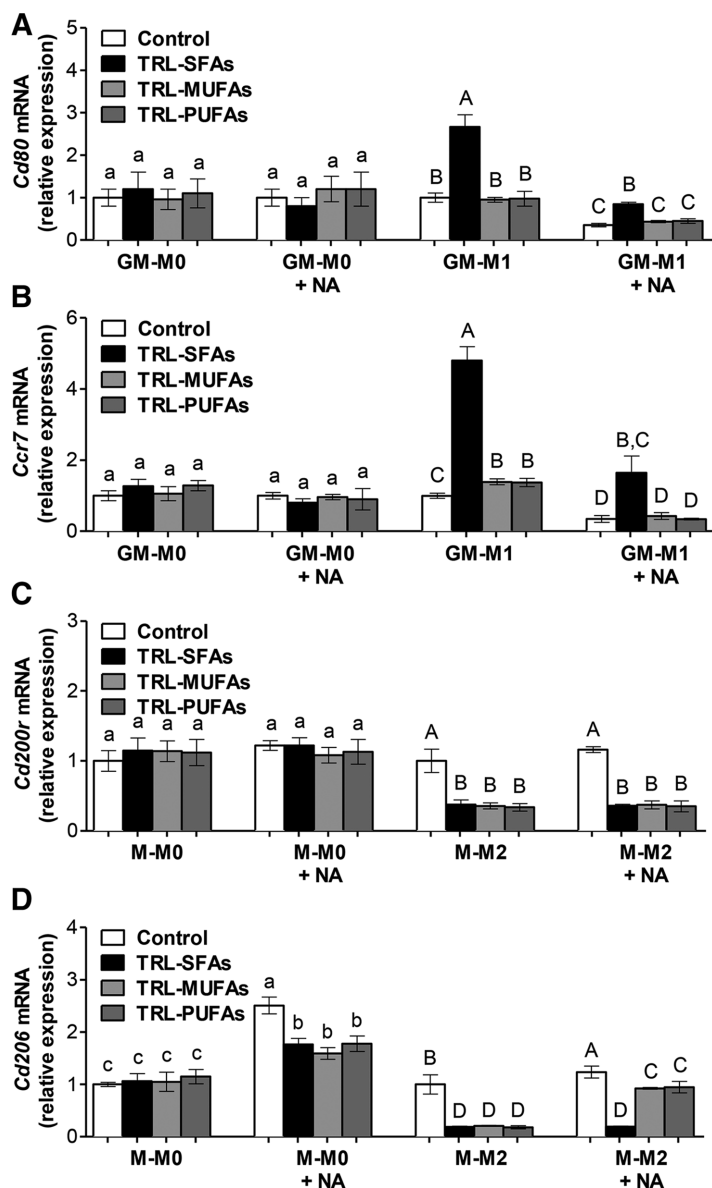
**Figure 1.** Mitochondrial activity (A) and its influence by postprandial TRLs and niacin (NA) in human GM-M0, GM-M1, M-M0, and M-M2 macrophages. (B) GM-M0 and GM-M1 macrophages in the presence or absence of postprandial TRLs; (C) GM-M1 macrophages, time-course in the presence or absence of postprandial TRLs and niacin; (D) M-M0 and M-M2 macrophages in the presence or absence of postprandial TRLs; (E) M-M2 macrophages, time-course in the presence or absence of postprandial TRLs and niacin; (F) GM-M1 and M-M2 in the absence or presence of niacin. Freshly human monocytes from six healthy male volunteers were differentiated with GM-CSF (50 ng/mL) or M-CSF (50 ng/mL) and then polarized with LPS (100 ng/mL) plus IFN- $\gamma$  (20 ng/mL) or IL-4 (20 ng/mL). By using GM-CSF or M-CSF, GM-M0, and GM-M1 or M-M0 and M-M2 macrophages were obtained. Cells were incubated with or without (control) autologous postprandial TRLs (100  $\mu$ g triglycerides/mL) and NA (100 nM). Postprandial TRLs were obtained from the above volunteers after the ingestion of oral fat emulsions (cow's milk cream: TRL-SFAs; refined olive oil: TRL-MUFAs; refined olive oil plus omega-3 LCPUFAs: TRL-PUFAs). Mitochondrial activity was determined by MTT assay. Values are expressed as the mean  $\pm$  SD of six independent experiments (one per volunteer) performed in triplicate. Data with different lowercase letters are significantly different at  $p < 0.05$  according to the one-way analysis of variance statistical analysis followed by Tukey post hoc test. Two-way ANOVA followed by a Bonferroni post hoc test was used when appropriate. # Significantly different with respect to NA treated cells.

laden with lipids through the uptake of atherogenic lipoproteins in the vascular intima is the hallmark of plaque development and its complications [20]. It is widely agreed that, compared to diets rich in SFAs or carbohydrates, diets with relatively high MUFA content may elicit benefits against cardiovascular risk outcomes. In the present study, we expand this knowledge and demonstrate that exogenous fatty acids in postprandial TRLs exhibit the capacity of regulating the monocyte-macrophage plasticity.

Macrophages are plastic cells whose physiological functions are at least in part dependent of their origin and their mi-

croenvironment. The classically activated macrophages (M1 phenotype) are considered as pro-inflammatory, whereas the alternatively activated macrophages (M2 phenotype) are considered as anti-inflammatory and involved in tissue repair [21].

We are aware that the M1/M2 nomenclature is an oversimplification and represents the extremes of a continuum of functional phenotypes in vivo, which may be considered a limitation. Nevertheless, our study reinforces the notion that GM-CSF and M-CSF influence on the differentiation process from monocytes to macrophages and their polarization

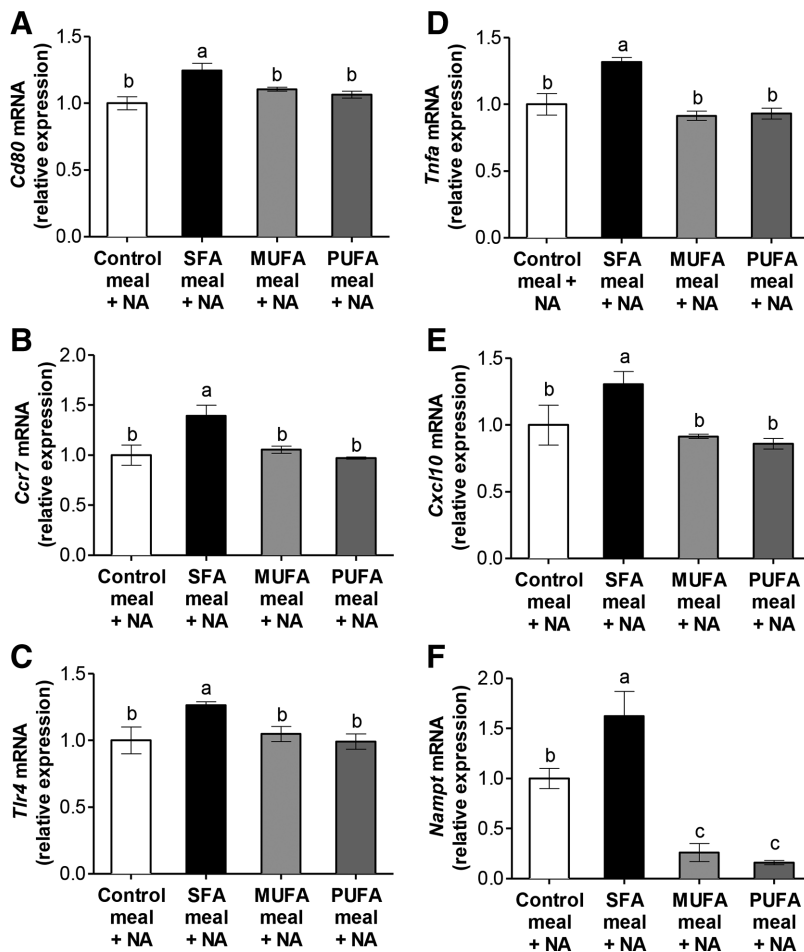


**Figure 2.** Expression of M1 and M2 gene markers in human GM-M0, GM-M1, M-M0, and M-M2 macrophages in the presence or absence of postprandial TRLs and niacin (NA). M1 gene markers: (A) *Cd80*; (B) *Ccr7*. M2 gene markers: (C) *Cd200r*; (D) *Cd206*. Freshly human monocytes from six healthy male volunteers were differentiated with GM-CSF (50 ng/mL) or M-CSF (50 ng/mL) and then polarized with LPS (100 ng/mL) plus IFN $\gamma$  (20 ng/mL) or IL-4 (20 ng/mL). By using GM-CSF or M-CSF, GM-M0 and GM-M1 or M-M0 and M-M2 macrophages were obtained. Cells were incubated with or without (control) autologous postprandial TRLs (100  $\mu$ g triglycerides/mL) and NA (100 nM). Postprandial TRLs were obtained from the above volunteers after the ingestion of oral fat emulsions (cow's milk cream: TRL-SFAs; refined olive oil: TRL-MUFAs; refined olive oil plus omega-3 LCPUFAs: TRL-PUFAs). Cells without TRL and NA treatment were used as control cells for data normalization/analysis. Values are expressed as the mean  $\pm$  SD of six independent experiments (one per volunteer) performed in triplicate. Data with different lowercase (GM-M0 and M-M0 macrophages) and uppercase (GM-M1 and M-M2 macrophages) letters are significantly different at  $p < 0.05$  according to the two-way analysis of variance statistical analysis followed by Bonferroni post hoc test.

[22–24], but differently. However, other authors did not find significant differences between GM-CSF and M-CSF stimulatory signals for the polarization of monocytes into M1 macrophages [25]. It is probable that these discrepancies are due to the experimental design, for example, the use of one type of cytokine for monocyte differentiation and then the use of another type of cytokine for macrophage polarization [26]. To our knowledge, the present study is the first to establish that GM-CSF and M-CSF do not equally promote human monocyte differentiation into naïve macrophages. Our study also shows that GM-CSF favors naïve macrophages more prone to M1 polarization, whereas M-CSF to M2 polarization.

Besides distinct functions and gene expression profiles of M1 and M2 macrophages, they exhibit contrasted metabolic

activities [27]. Metabolism in M1 macrophages is characterized by high levels of glycolysis and flux through the pentose phosphate pathway, and an elevation in certain Krebs cycle intermediates, which occurs in the matrix of the mitochondrion [28]. M1 macrophages also have increased synthesis of fatty acids requiring increased energy expenditure and therefore increased mitochondrial activity. Our study confirmed the higher mitochondrial activity in M1 macrophages than in M2 macrophages and more importantly that postprandial TRLs promote, probably as a source of fatty acids, the mitochondrial activity in M1 macrophages but not in naïve macrophages or M2-polarized macrophages. Mitochondrial activity has been implicated as a cause of aging, and mitochondrial dysfunction and reactive oxygen species production have been linked to neurodegeneration, cancer, and autoimmunity [29].

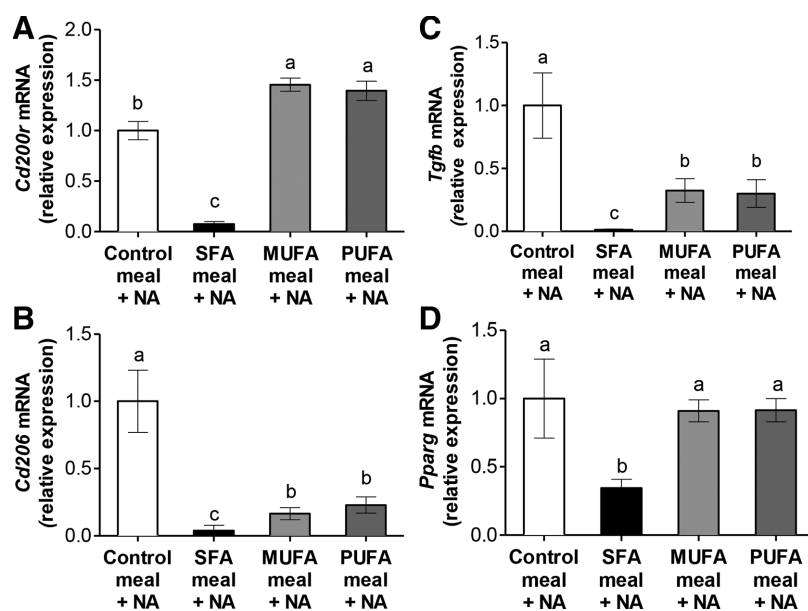


**Figure 3.** Expression of M1 gene markers in GM-M0 macrophages from human postprandial monocytes after the co-ingestion of oral fat emulsions and a single-dose of immediate-release niacin (NA). (A) *Cd80*; (B) *Ccr7*; (C) *Tlr4*; (D) *Tnfa*; (E) *Cxcl10*; (F) *Nampt*. Freshly human postprandial monocytes from six healthy male volunteers were isolated after the ingestion of oral fat emulsions rich in cow's milk cream (SFA meal), refined olive oil (MUFA meal), refined olive oil plus omega-3 LCPUFAs (PUFA meal), or a non-fat (control) meal in combination with a single-dose of immediate-release NA. Cells were then differentiated with GM-CSF (50 ng/mL) into GM-M0 macrophages. Values are expressed as the mean  $\pm$  SD of six independent experiments (one per volunteer) performed in triplicate. Data with different lowercase letters are significantly different at  $p < 0.05$  according to the one-way analysis of variance statistical analysis followed by Tukey post hoc test.

Noteworthy, niacin did not affect the mitochondrial activity in our model cells but buffered the postprandial-TRL-induced mitochondrial activity in M1 macrophages, unveiling novel beneficial effects of niacin in regulating metabolic functions of selective macrophage subtypes.

Gene expression markers for GM-M1 and M-M2 macrophages were analyzed in the absence or presence of autologous postprandial TRLs and niacin. Our data showed that postprandial TRL-SFAs skewed naïve macrophages toward M1-like macrophages, whereas M2 macrophage gene markers were compromised, contrarily to that observed with postprandial TRL-MUFAs and TRL-PUFAs, skewing naïve macrophages toward M2-like macrophages. These findings are in accordance with previous studies in which SFAs, via TLR2, TLR4, and NF- $\kappa$ B activation, were shown to be inducers of M1 macrophages, an effect that was inhibited by MUFAs and PUFAs [30–34]. In our study, we provide fatty acids in a more physiological way by using postprandial TRLs. It was interesting to observe that niacin had an inhibitory role to modulate the postprandial-TRL-induced macrophage polarization into M1 macrophages and had an activator role to modulate the macrophage polarization into M2 macrophages when co-incubated with postprandial TRLs

rich in MUFAs. These findings were consistent with the release of pro-inflammatory and anti-inflammatory cytokines and demonstrate that the type of predominant fatty acids in the diet could have a critical role in the plasticity of human macrophages in vitro. Dietary SFAs acutely induced pro-inflammatory macrophages, whereas niacin and dietary MUFAs and omega-3 LCPUFAs favoured the other extreme of a dynamic changing state of macrophages at different levels of activation represented by anti-inflammatory macrophages. In addition, we analyzed gene expression of M1 and M2 markers in naïve M-M0 macrophages obtained from postprandial monocytes of healthy volunteers subjected to SFA, MUFA, and PUFA meals in combination with a single-dose of immediate-release niacin. Immediate-release niacin is usually completely absorbed within 1–2 h, whereas dietary fatty acids are largely incorporated into postprandial TRLs and released into the blood stream with a peak occurring 2–3 h after fatty food consumption. Therefore, when co-ingested together, postprandial TRLs and niacin may coexist with circulating monocytes prior to their migration to vascular endothelium and differentiation to macrophages. Furthermore, postprandial TRL are irreversibly trapped (in contrast to LDL, which are reversibly trapped) in the subendothelial space [35]



**Figure 4.** Expression of M2 gene markers in M-M0 macrophages from human postprandial monocytes after the co-ingestion of oral fat emulsions and a single-dose of immediate-release niacin (NA). (A) *Cd200r*; (B) *Cd206*; (C) *Tgfb*; (D) *Pparg*. Freshly human postprandial monocytes from six healthy male volunteers were isolated after the ingestion of oral fat emulsions rich in cow's milk cream (SFA meal), refined olive oil (MUFA meal), refined olive oil plus omega-3 LCPUFAs (PUFA meal), or a non-fat (control) meal in combination with a single-dose of immediate-release NA. Cells were then differentiated with M-CSF (50 ng/mL) into M-M0 macrophages. Values are expressed as the mean  $\pm$  SD of six independent experiments (one per volunteer) performed in triplicate. Data with different lowercase letters are significantly different at  $p < 0.05$  according to the one-way analysis of variance statistical analysis followed by Tukey post hoc test.

where they, and niacin, may interact with surrounding cells, including macrophages [36]. In support of this notion, the human monocyte-macrophage lineage is well equipped with genes encoding receptors that recognize postprandial TRLs [11, 36] and niacin [2, 37]. Also relevant, a regulatory crosstalk and interference between predominant fatty acids from postprandial TRLs and niacin pathways have been recently reported in human myeloid cells [38]. The mechanistic basis of these effects remains unclear. In the present study, we have further established that high-fat meals primed the postprandial monocytes in healthy volunteers to differentiate into naïve macrophages with predisposition to polarize into M1-like macrophages (SFA meal) or into M2-like macrophages (MUFA meal, PUFA meal) in the setting of immediate-release niacin treatment, which measure the physiological relevance of our *in vitro* findings on the role of dietary fatty acids in postprandial TRLs in the plasticity of human monocyte-macrophage axis.

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S.M., R.A., F.J.G.M., and S.L. designed the study. D.R., M.P.C., and M.C.N. performed *in vitro* studies. S.M., B.B., and S.L. performed human intervention. S.M., F.J.G.M., and S.L. interpreted the data and wrote the paper. All authors discussed the results and implications and commented on the manuscript at all stages.

The authors have declared no conflict of interest.

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