Food Chemistry: Molecular Sciences 5 (2022) 100133

Contents lists available at ScienceDirect

Food Chemistry: Molecular Sciences

journal homepage: www.sciencedirect.com/journal/food-chemistry-molecular-sciences

New evidence for dietary fatty acids in the neutrophil traffic between the bone marrow and the peripheral blood

Almudena Ortega-Gomez [a,b], Sergio Lopez [a,c,d], Lourdes M. Varela [a,d,e], Sara Jaramillo [a], Francisco J.G. Muriana [a], Rocio Abia [a,*]

[a] Laboratory of Cellular and Molecular Nutrition, Instituto de la Grasa, The Spanish National Research Council (CSIC), 41013 Seville, Spain
[b] Department of Cellular and Molecular Endocrinology, Instituto de Investigacion Biomedica de Malaga (IBIMA), Virgen de la Victoria University Hospital, 29010 Malaga, Spain
[c] Department of Cell Biology, Faculty of Biology, University of Seville, 41012 Seville, Spain
[d] Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocio/CSIC/University of Seville, 41013 Seville, Spain
[e] Department of Medical Physiology and Biophysics, School of Medicine, University of Seville, 41009 Seville, Spain

ARTICLE INFO

Keywords:
Olive oil
Butter
Dietary fatty acids
High-fat diets
Neutrophil mobilisation
Bone marrow inflammation

ABSTRACT

Chronic administration of a high-fat diet in mice has been established to influence the generation and trafficking of immune cells such as neutrophils in the bone marrow, the dysregulation of which may contribute to a wide range of diseases. However, no studies have tested the hypothesis that a short-term, high-fat diet could early modulate the neutrophil release from bone marrow at fasting and at postprandial in response to a high-fat meal challenge, and that the predominant type of fatty acids in dietary fats could play a role in both context conditions. Based on these premises, we aimed to establish the effects of different fats [butter, enriched in saturated fatty acids (SFAs), olive oil, enriched in monounsaturated fatty acids (MUFAs), and olive oil supplemented with eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids] on neutrophil navigation from bone marrow to blood in mice. The analysis of cellular models for mechanistic understanding and of postprandial blood samples from healthy volunteers for translational purposes was assessed. The results revealed a powerful effect of dietary SFAs in promotion the neutrophil traffic from bone marrow to blood via the CXCL2-CXCR2 axis. Dietary SFAs, but not MUFAs or EPA and DHA, were also associated with increased neutrophil apoptosis and bone marrow inflammation. Similar dietary fatty-acid-induced postprandial neutrophilia was observed in otherwise healthy humans. Therefore, dietary MUFAs might preserve bone marrow health and proper migration of bone marrow neutrophils early in the course of high-fat diets even after the intake of high-fat meals.

1. Introduction

Granulopoiesis is the process by which neutrophils develop to maturation from pluripotent hematopoietic stem cells (HSCs) in the bone marrow (Yvan-Charvet & Ng, 2019). Neutrophils are the most abundant immune cells in the mammalian system and the first immune cells in responding to inflammation, infection or other stresses by their movement from bone marrow to bloodstream aiming for maintenance of homeostasis (Singhal & Kumar, 2021). Bone marrow production, storage and release of neutrophils are not only decisive in the correct function of both innate and adaptive immune systems (De Filippo & Rankin, 2020), but also in the regeneration of bone marrow niches and regulation of the numbers and function of bone marrow HSCs in a feedback pathway (Cai et al., 2021). An increased neutrophil count in blood has been shown to be associated with the pathogenesis of a broad spectrum of human inflammatory disorders, including autoimmune diseases (Petrelli et al., 2022), heart failure (Sauer et al., 2022) and cancer (Poto et al., 2022). Therefore, the identification of factors

Abbreviations: HFDs, high-fat diets; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; HSCs, hematopoietic stem cells; PUFAs, polyunsaturated fatty acids; OFLs, oral fat loads; OFMs, oral fat meals; LFD, low-fat diet; OSL, oral saline load; MMP9, matrix metalloproteinase 9; TRLs, triglyceride-rich lipoproteins; BMSF, bone marrow supernatant fluid; HBSS, Hank's balance salt solution; FACS, fluorescence-activated cell sorting; MFI, mean fluorescence intensity; FSC, forward scatter; SSC, side scatter; PI, propidium iodide; OCM, oral control meal; qRT-PCR, quantitative real-time reverse transcription polymerase chain reaction; Ct, threshold cycle.

* Corresponding author: Instituto de la Grasa (CSIC), Campus Universitario Pablo de Olavide (UPO), Edificio 46, Ctra. de Utrera Km 1, 41013 Sevilla, Spain.
E-mail address: abia@ig.csic.es (R. Abia).

https://doi.org/10.1016/j.fochms.2022.100133
Received 8 July 2022; Received in revised form 2 September 2022; Accepted 3 September 2022
Available online 6 September 2022

involved in bone marrow production and how they drive bone marrow storage or release of neutrophils remain challenging issues that require further investigation.

It is well known that long-term exposure to high-fat diets (HFDs), often rich in saturated fatty acids (SFAs), induces weight gain and obesity. This chronic condition is one of the major public health concerns worldwide that accounts for morbidity and premature death, and commonly drives a state of inflammation linked with a defective immune system (Uribe-Querol & Rosales, 2022). Many authors have investigated the role of neutrophils from bone marrow in the malfunction of white and brown adipocytes, including a recent review on the interplay between SFAs and specific innate immune receptors in the regulation of the obesity-induced adipose tissue inflammation, while unsaturated fatty acids [including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] prevent the effects of SFAs (Hachiya, Tanaka, Itoh, & Suganami, 2022). The consumption of diets rich in monounsaturated fatty acids (MUFAs) has also been shown to decrease the occurrence of obesity and the risk of inflammation (Ravaut, Legiot, Bergeron, & Mounier, 2021). In addition, the concept that HFDs administered for a long-term period play a role in the generation and trafficking of immune cells in the bone marrow of mice has been recently established (Lemus-Conejo et al., 2021, Medrano, Lemus-Conejo, Lopez, Millan-Linares, Rosillo, Muñiz, Calderon, Jaramillo, Perez-Simon, Muriana, & Abia, 2022). HFDs enriched in SFAs or in MUFAs induced contrasting effects on hematopoietic cellular architecture in the bone marrow and the peripheral blood of mice with obesity. While the numbers of neutrophils increased in both compartments by MUFAs, a decrease of neutrophils was reported in the bone marrow by SFAs. Furthermore, there is available evidence to suggest that the chemokine receptors CXCR2 and CXCR4 and their ligands CXCL2 and CXCL12 antagonistically govern the instructions for bone marrow storage or release of neutrophils (De Filippo & Rankin, 2018, Eash et al., 2010, Sawant et al., 2021). However, these observations still leave many open questions on molecular pathways involved in the early stages before the onset of HFD-induced obesity, particularly whether dietary fatty acids for a short-term period or acutely can impact on CXCR2-CXCL2 and/or CXCR4-CXCL12 axes for neutrophil production, storage or release at the bone marrow level. This novel understanding may provide further insight into the mechanisms by which inflammatory regulation in the development of obesity is important to avoid co-morbidities, such as diabetes, acute kidney injury, hepatic steatosis and atherosclerosis (Furman et al., 2019).

Thus, our aim was to establish the effects and mechanisms of different fats (butter, enriched in SFAs, olive oil, enriched in MUFAs, and olive oil supplemented with EPA and DHA) when administered in HFDs for a short-term period or by gavage on bone marrow neutrophil mobilisation and inflammatory state in mice. The release of neutrophils into the circulation was also postprandially explored in healthy volunteers. We postulated that an early modulation of neutrophil behaviour from bone marrow and of other events related to bone marrow inflammation could be dependent on the predominant type of fatty acids in dietary fats through the CXCR2-CXCL2 or the CXCR4-CXCL12 signalling axis.

2. Materials and methods

2.1. Animals and dietary fats

The animal study was designed following the ARRIVE guidelines for the randomization, execution of experiments and reporting data. All animal procedures were performed according to ethical regulations formulated in the Spanish law and European legislation on the care and use of experimental animals (RD 53/2013; EU Directive of 2010/63/EU and 2012/707/EU). Protocols were approved by the Ethics Committees at University Pablo de Olavide and CSIC (03/02/2017/030). Six-week-old wild-type C57BL/6J male mice (Charles River Lab, Ecully, France) were housed in a specific pathogen-free facility at ~22 °C on a 12 h light/dark cycle with daily care by the animal facility of the Centro Andaluz de Biologia del Desarrollo (CABD, Seville). Animals (72 in total) were fed a chow diet (here referred as low-fat diet, LFD) [75.6 % energy from carbohydrates, 13.6 % energy from proteins and 10.7 % energy from fats (soybean oil)] ad libitum and had free access to drinking water for 2 weeks to settle in. Then, mice were randomly assigned to one of four treatment diets (n = 18/group): the LFD (control) and three isocaloric HFDs based on the LFD with additional 43 % energy as fat from anhydrous butter, as a source of SFAs (HFD-SFAs); olive oil, as a source of MUFAs (HFD-MUFAs); or olive oil (40 % energy) plus EPA + DHA (3 % energy) in the form of ethyl esters, as a source of MUFAs and long-chain omega-3 PUFAs (HFD-MUFAs + EPA + DHA) (Supplemental Table 1) for 4 weeks. All diets were prepared by Panlab Laboratories (SAFE, Augy, France). Doses of anhydrous butter and olive oil were chosen to represent reasonable amounts of SFAs and MUFAs expected to be present in current human diets: SFA-rich Western diet and MUFA-rich Mediterranean diet (Estruch et al., 2013). The dose of EPA + DHA was equivalent to that used in clinical trials (Bradberry & Hilleman, 2013). Diets and drinking water were refreshed every day. Olive oil was devoid of minor constituents as obtained by physical refining of virgin olive oil in a discontinuous deodorizer that used nitrogen as stripping gas at the Core Facilities for Oil Extraction and Refining of the Instituto de la Grasa (Seville). The fatty acid composition of the diets (Supplemental Table 2) was determined via lipid extraction, saponification and capillary gas chromatography (Montserrat-de la Paz et al., 2016). At the end of the 4-week feeding period, mice were kept on fasting for 12 h. Nine mice per group were euthanized with an overdose of pentobarbital (1:10 in PBS, 150 mg/kg intraperitoneal injection), blood was collected by cardiac puncture using heparinized syringes and tubes (MiniCollect 1 mL K_3EDTA, Grenier Bio-One, Kremsmünster, Austria), then femur and tibia were removed.

For the oral gavage study, the remaining nine fasted mice of each group were administered with 300 μL of 0.9 % sodium chloride (oral saline load, OSL) or the same volume of melted anhydrous butter (oral fat load-SFAs, OFL-SFAs), olive oil (OFL-MUFAs) or olive oil (280 μL) plus EPA + DHA (20 μL containing 8.5 mg EPA and 7 mg DHA in the form of ethyl esters) (OFL-MUFAs + EPA + DHA). After 3 h of gavage, which corresponded to the time of maximum level of TGs in blood (data not shown), mice were euthanized and blood and bones were collected as above and used for further analysis.

2.1.1. Biochemical analyses

Serum levels of TGs and CXCL2 were measured by a colorimetric assay kit (Bio Science Medical, Madrid, Spain) and an ELISA kit (Abcam, Cambridge, UK), respectively. Serum and bone marrow supernatant fluid (BMSF) levels of IL-1β, IL-6 and TNF-α were assessed according to the protocol provided by the manufacturer Bio-Plex Pro Reagent Kit (Bio-Rad Laboratories, Hercules, CA, USA).

2.1.2. Obtention of bone marrow cells and BMSF, and isolation of bone marrow neutrophils

Cells from bone marrow and BMSF of wild-type C57BL/6J male mice fed with HFDs or administered with OFLs were isolated from each group of mice. Femurs and tibiae were aseptically removed and dissected free of adherent soft tissue. Details of protocols can be found in the Supplemental Material.

2.1.3. Fluorescence-activated cell sorting (FACS) analysis

Surface staining for FACS analysis, either in blood samples after erythrocyte lysis or in isolated bone marrow neutrophils from mice, was performed using antibodies [CD11b-PerpCy5.5 (Becton Dickinson, Madrid, Spain), Ly6C-FITC (Miltenyi Biotec, Madrid, Spain), Ly6G-PE (eBioscience, San Diego, CA, USA) and CXCR4-APC (Becton Dickinson, Madrid, Spain)] in PBS with 0.1 % BSA for 30 min in the dark at room temperature. After labelling, fluorescence intensity was measured by a

FACSCanto II Cell Analyser and calibrated using FACSCanto II Cell software (Becton Dickinson, Madrid, Spain). Mean fluorescence intensity (MFI) of 10,000 counted cells was measured in each sample. Leukocytes were gated on high forward scatter (FSC^{high}) and high side scatter (SSC^{high}). Neutrophils were defined using CD11b versus Ly6G. Non-specific Fc-receptor binding of antibodies was blocked by addition of an anti-CD16/CD32 antibody (eBioscience, San Diego, CA, USA). Absolute number of neutrophils was determined with Flow-Count Fluorospheres (Beckman Coulter, Brea, CA, USA).

2.1.4. Apoptosis assay

Apoptosis in isolated mouse bone marrow neutrophils was assessed by FACS analysis using Annexin V-FITC kit (Miltenyi Biotec, Madrid, Spain) after incubation with staurosporine (200 nM; Tocris Bioscience, Bristol, UK) or human postprandial TRLs (100 μg of TGs/mL) (see below) for 12 h. Apoptotic cells were labelled with Annexin V conjugated to green-fluorescent Alexa Fluor 488 dye and necrotic cells were labelled with red-fluorescent propidium iodide (PI) (Thermo Fisher Scientific, Madrid, Spain). These populations were evaluated using FACSCanto II Cell Analyser (Becton Dickinson, Madrid, Spain) with an excitation wavelength of 488 nm and a 530 nm filter for the detection of Alexa Fluor 488 and a 585 nm filter for the detection of PI. The data were analysed using FACSDiva software (Becton Dickinson, Madrid, Spain). At least 10,000 events for each sample were analysed and gated according to light scattering properties.

2.2. Human study

All protocols were approved before the start of the study by the Human Clinical Commission and the Ethics Committee of Hospitales Universitarios Virgen del Rocio (HHUUVR, Seville) (ES41091008015) and informed consent was obtained from each participant. The study was conducted according to the principles outlined in the Helsinki Declaration of the World Medical Association and in line with the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals. Twelve male volunteers, aged 25 to 35 years, non-smokers, with no medical history of disease known, abnormality of haematological or biochemical parameters were recruited. The study was conducted as a within-subjects crossover design in which the participants attended the Clinic Experimental Research Unit for Vascular Risk at the HHUUVR for a total of eight different occasions. In a random order, on four occasions the day prior to initiate a 1-week prechallenge feeding period on planned menus with the inclusion of soybean oil (control diet), anhydrous butter (SFA diet), olive oil (MUFA diet) or olive oil plus a daily dose of long-chain omega-3 PUFAs (460 mg of EPA and 380 mg of DHA in the form of ethyl esters) (MUFA + EPA + DHA diet) as principal source of fat in the diet. To assess baseline status, blood samples were collected after 12 h of fasting. On four additional occasions, the participants were summoned 1-week later to collect blood samples at fasting (12 h) and then to initiate the postprandial study. For that purpose, fasted participants from control diet or SFA, MUFA and MUFA + EPA + DHA diets were respectively given a meal without fat (oral control meal, OCM) or OFMs containing anhydrous butter (OFM-SFAs), olive oil (OFM-MUFAs) or olive oil plus a dose of long-chain omega-3 PUFAs (920 mg of EPA and 760 mg of DHA in the form of ethyl esters) (OFM-MUFAs + EPA + DHA). There was an interval of at least 1 week between each complete intervention. Each participant served as his own control. OFMs were prepared according to the method described by the Patent WO/2014/191597 (Bermudez et al., 2014) with water, sucrose (30 g/m^2 of body surface area), fat (50 g/m^2 of body surface area), emulsifier and flavouring. After 2 h of the ingestion of the meals, which corresponded to the time of maximum level of TGs in blood (data not shown), blood was collected in either standard tubes (blood samples), sodium citrate (final concentration, 0.129 mmol/L) tubes (plasma samples) or gel Z tubes (serum samples) (Sarstedt, Numbrecht, Germany). Blood neutrophil counts were analysed using Coulter Hematology Analyser (Beckman Coulter, Brea, CA, USA).

2.2.1. Biochemical analyses

Plasma levels of TGs and matrix metalloproteinase 9 (MMP9) were measured with a Hitachi Modular Analytics D-2400 analyser (Roche Diagnostics, Basel, Switzerland) and an ELISA kit (R&D Systems, Abingdon, UK), respectively. The levels of MMP9 mRNA in blood neutrophils were determined as described below.

2.2.2. Preparation and analysis of postprandial TG-rich lipoproteins

Postprandial TG-rich lipoproteins (TRLs) were isolated from serum samples after the intake of OFMs (Montserrat-de la Paz et al., 2016) to obtain TRL-SFAs, TRL-MUFAs and TRL-MUFAs + EPA + DHA. Each type of postprandial TRLs was pooled, dialysed against cold PBS and immediately stored at –80 °C. Only once-thawed, postprandial TRL samples were used for cell culture studies. The fatty acid composition in postprandial TRLs was determined as previously described (Montserrat-de la Paz et al., 2017) (Supplemental Table 3). Details of the analysis can be found in the Supplemental Material.

2.3. RNA isolation and quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) analysis

Total RNA from murine bone marrow cells, SVEC4-10 cells, bone marrow neutrophils and human circulating neutrophils was isolated by TRisure Reagent (Bioline GmbH, Berlin, Germany) and purified through spin columns of the Direct-zol MiniPrep Kit (Zymo Research, Irvine, CA, USA). The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and 18 s housekeeping genes were used for normalization. Sequences for qRT-PCR primers can be found in Supplemental Table 4. Details of the protocol can be found in the Supplemental Material.

2.4. Neutrophil trans-lymphatic endothelial migration assay

SVEC4-10 lymphatic endothelial-like cells (ATCC, Manassas, VA, USA) were grown to confluency on the upper surface of transwell inserts (3-μm pore size) (Corning, Kenneburg, ME, USA), which were previously coated with collagen I (Merck Millipore, Barcelona, Spain). Details of the protocol can be found in the Supplemental Material.

2.5. Statistical analysis

Statistical analysis was performed with GraphPad Prism 6 software (GraphPad Software, La Jolla, CA, USA). All data are expressed as mean ± SD. Three technical replicates per sample were run in each independent experiment. Unpaired Student *t*-test, Mann-Whitney test or Kruskal-Wallis test with post hoc Dunn test was used as appropriate. Statistical significance was set at a level of $p < 0.05$.

3. Results

3.1. Influence of HFDs and OFLs on serum TGs in C57BL/6J mice

Acute hypertriglyceridemia was investigated after the administration of OFLs (rich in SFAs, MUFAs or MUFAs + EPA + DHA) to fasting C57BL/6J mice, which had been previously fed with corresponding HFDs (rich in SFAs, MUFAs or MUFAs + EPA + DHA) for 4 weeks. A fourth group of animals was given a LFD (control for HFD effects) followed by an OSL (control for OFL effects). At fasting, HFD-SFAs (195.3 ± 7.5 mg/dL) and HFD-MUFAs (152.6 ± 13.1 mg/dL) increased ($p < 0.05$) serum TGs when compared to LFD (129.3 ± 10.8 mg/dL) or HFD-MUFAs + EPA + DHA (132.1 ± 19.0 mg/dL). The effect of the HFD-SFAs was greater ($p < 0.05$) than that found with the HFD-MUFAs. At 3-h postprandial, the values of serum TGs increased up to 378.0 ± 45.7 mg/dL after the OFL-SFAs, 349.9 ± 24.6 mg/dL after the OFL-MUFAs and 273.6 ± 28.1 mg/dL after the OFL-MUFAs + EPA + DHA. These

values did not statistically differ between OFL-SFAs and OFL-MUFAs. Unsurprisingly, the serum TG concentration was postprandially unchanged (123.3 ± 20.9 mg/dL, mean value over a 6-h postprandial period) by the OSL in the LFD group.

3.2. Influence of HFDs and OFLs on circulating and bone marrow neutrophil numbers in C57BL/6J mice

FACS analysis was first conducted on blood and bone marrow samples of mice fed HFDs and challenged with OFLs to allow detection of neutrophils at fasting and at postprandial, respectively (Fig. 1**A**). At the end of 4 weeks on HFDs, animals showed a higher number of circulating neutrophils than that found in animals on LFD at fasting (Fig. 1**B**). A more pronounced effect was observed with the HFD-SFAs. The same animals submitted to OFLs exhibited similar profiles in the number of circulating neutrophils at postprandial as at fasting, but those at postprandial were more elevated than those at fasting (Fig. 1B). Of note, postprandial levels of circulating neutrophils did not correlate with postprandial levels of circulating TGs ($r = 0.8919$; $p = 0.2989$), which may suggest a specific role of the type of dietary fatty acids in OFLs on the pool of neutrophils in the blood compartment. Looking at bone marrow, profiles in the number of neutrophils at fasting mirrored those found in the circulation, with only the HFD-SFAs reaching statistical difference (Fig. 1**C**). However, bone marrow compartment was depleted

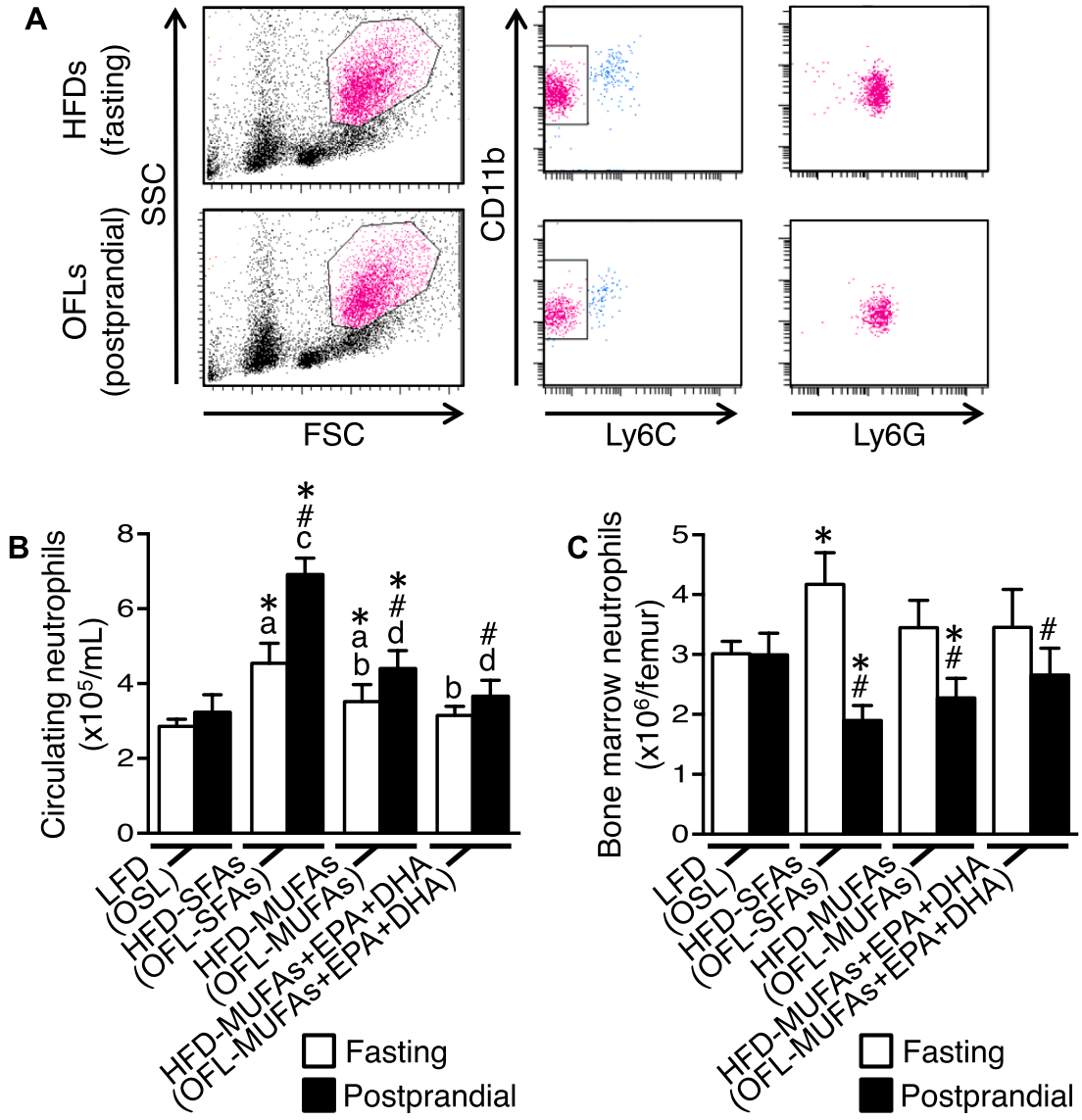

Fig. 1. The type of dietary fat in high-fat diets (HFDs) and oral-fat loads (OFLs) modulates the number of neutrophils in the blood and bone marrow of C57BL/6J mice. Animals were fed a low-fat diet (LFD) or HFDs containing anhydrous butter (HFD-SFAs), olive oil (HFD-MUFAs) or olive oil plus EPA + DHA (HFD-MUFAs + EPA + DHA) for 4 weeks. Blood and bone marrow were obtained at fasting (at the end of the diet period, $n = 9$) and at postprandial (after the administration of the oral loads, $n = 9$). (**A**) Representative gating strategy and dot plots (SSC/FSC and CD11b/Ly6G) of neutrophils in the blood or bone marrow. Ly6C (central panels) was used as a marker to discriminate monocytes (blue dots) from neutrophils (pink dots). (**B**) Number of circulating neutrophils at fasting (white), and at postprandial after the animals received an oral-saline load (OSL) or an OFL of melted anhydrous butter (OFL-SFAs), olive oil (OFL-MUFAs) or olive oil plus EPA + DHA (OFL-MUFAs + EPA + DHA) (black). (**C**) Number of bone marrow neutrophils at fasting (white) and at postprandial (black). For all graphs, the bar heights reflect the mean and the error bars represent *SD* of nine independent biological replicates. *Significant difference of HFD or OFL compared to LFD or OSL. #Significant difference of postprandial value compared to fasting value within each dietary fat. Different letters assigned to fasting values (a and b) or to postprandial values (c and d) indicate significant differences between HFDs or OFLs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of neutrophils at postprandial, more markedly in animals after the OFL-SFAs (Fig. 1C).

3.3. Influence of HFDs, OFLs and TRLs on bone marrow neutrophil apoptosis in C57BL/6J mice

We next evaluated survival properties in freshly isolated bone marrow neutrophils in mice at fasting (at the end on HFDs) and at postprandial (following OFLs). An increased apoptosis was only observed in bone marrow neutrophils at fasting from mice fed with the HFD-SFAs (Fig. 2**A**). While the OFL-MUFAs and OFL-MUFAs + EPA + DHA did not further affect the viability of neutrophils, the OFL-SFAs increased apoptotic neutrophils in bone marrow at postprandial to a level even higher than that observed at fasting (Fig. 2A). These findings

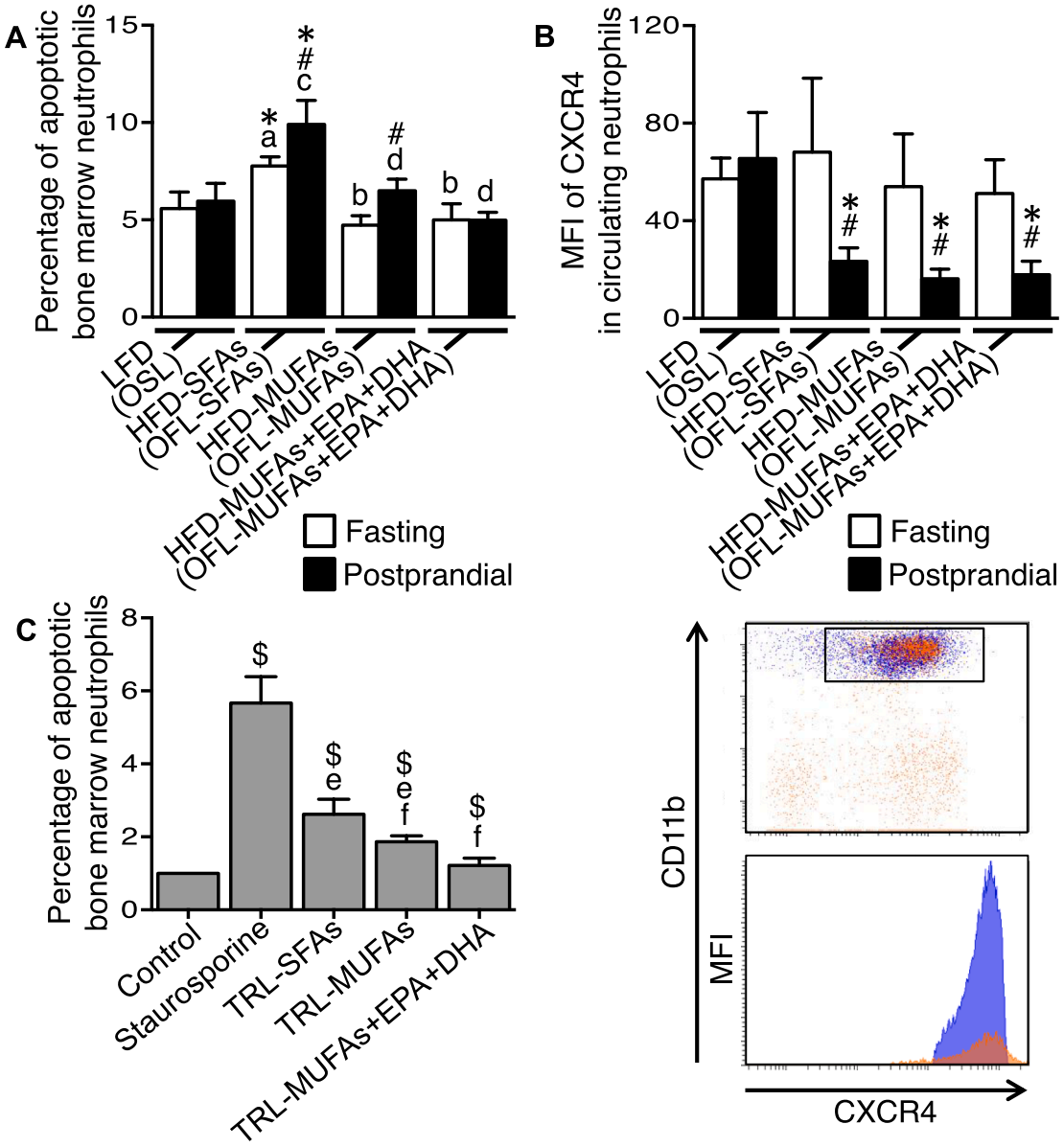

Fig. 2. The type of dietary fat in high-fat diets (HFDs), oral-fat loads (OFLs) and postprandial triglyceride-rich lipoproteins (TRLs) modulates the level of apoptotic bone marrow neutrophils of C57BL/6J mice. Animals were fed a low-fat diet (LFD) or HFDs containing anhydrous butter (HFD-SFAs), olive oil (HFD-MUFAs) or olive oil plus EPA + DHA (HFD-MUFAs + EPA + DHA) for 4 weeks. Blood and bone marrow were obtained at fasting (at the end of the diet period, $n = 9$) and at postprandial (after the administration of the oral loads, $n = 9$). (**A**) Percentage of apoptotic bone marrow neutrophils at fasting (white), and at postprandial after the animals received an oral-saline load (OSL) or an OFL of melted anhydrous butter (OFL-SFAs), olive oil (OFL-MUFAs) or olive oil plus EPA + DHA (OFL-MUFAs + EPA + DHA) (black). (**B**) Mean fluorescence intensity (MFI) of CXCR4 in circulating neutrophils at fasting (white) and at postprandial (black). In the lower panel are reported merged representative dot plot and FACS histogram analyses of neutrophil CXCR4 expression in blood samples at fasting (purple) and at postprandial (orange). (**C**) Relative percentage of apoptotic mouse bone marrow neutrophils after incubation with staurosporine (200 nM) or human postprandial TRLs (100 μg of TGs/mL) for 12 h. The different TRLs were isolated from postprandial serum samples of healthy volunteers after the intake of oral-fat meals containing anhydrous butter (TRL-SFAs), olive oil (TRL-MUFAs) or olive oil plus a dose of EPA + DHA (TRL-MUFAs + EPA + DHA). For all graphs, the bar heights reflect the mean and the error bars represent *SD* of nine independent biological replicates. *Significant difference of HFD or OFL compared to LFD or OSL. #Significant difference of postprandial value compared to fasting value within each dietary fat. Different letters assigned to fasting values (a and b) or to postprandial values (c and d) indicate significant differences between HFDs or OFLs. $Significant difference of the treatment compared to untreated (control) cells. Different letters (e and f) indicate significant differences between postprandial TRLs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

are unlikely due to the homing of senescent neutrophils back to the bone marrow, as CXCR4 expression in circulating neutrophils was not affected at fasting by HFDs or was repressed at postprandial by OFLs (Fig. 2**B**, **upper panel**). Representative FACS analysis for CXCR4 expression in circulating neutrophils is shown in the lower panel. Furthermore, we explored ex vivo whether postprandial TRLs isolated from healthy volunteers that ingested meals enriched in SFAs, MUFAs or MUFAs + EPA + DHA could directly induce apoptosis in neutrophils from bone marrow of mice fed with the LFD. Similar to results in vivo, bone marrow neutrophils entered into apoptosis due to postprandial TRL treatment (TRL-SFAs ≥ TRL-MUFAs ≥ TRL-MUFAs + EPA + DHA = control) (Fig. 2**C**). TRL-SFAs had half the potency of staurosporine.

3.4. Influence of HFDs and OFLs on inflammatory markers in BMSF and blood in C57BL/6J mice

Using bio-plex assays, we measured the concentration of IL-1β, IL-6 and TNF-α in BMSF and serum at fasting and at postprandial. None of the inflammatory cytokines, except TNF-α that increased in the BMSF of mice fed with the HFD-SFAs, were altered at fasting at the end on HFDs (Fig. 3**A-3F**). However, all of the inflammatory cytokines were increased in BMSF and serum at postprandial following OFLs (OFL-SFAs > OFL-MUFAs = OFL-MUFAs + EPA + DHA) (Fig. 3A-3F). These data suggest that bone marrow inflammation paired up with circulating inflammatory cytokines could be transiently induced by the intake of dietary fatty acids, especially in response to SFAs.

3.5. Potential mechanisms behind the influence of HFDs and OFLs on bone marrow neutrophil numbers in C57BL/6J mice

The chemokine CXCL2, which exerts its function via the CXCR2 receptor, is known to mobilize neutrophils to transit from bone marrow to peripheral tissues in response to inflammation and/or infection, and also to contribute to the potency of neutrophil functions and to restore homeostasis at the injured site (Sawant et al., 2021). Therefore, we decided to investigate the impact of HFDs and OFLs on this axis by the transcriptional analysis of genes encoding CXCL2 in bone marrow cells and CXCR2 in bone marrow neutrophils at fasting and at postprandial. Following qRT-PCR, there was an increase at fasting in the levels of CXCL2 mRNA in bone marrow cells of mice fed with the HFD-SFAs and HFD-MUFAs when compared to the LFD or the HFD-MUFAs + EPA + DHA (Fig. 4**A**). This effect was also observed at postprandial after the administration of the all OFLs, but more dramatically with the OFL-SFAs (Fig. 4A). Since CXCL2 is constitutively and highly expressed in the bone marrow endothelium (Eash et al., 2010) and the primary site for binding and uptake of postprandial TRLs in the bone marrow is its sinusoidal endothelium (Niemeier et al., 2008), we next examined the potential of postprandial TRLs concerning the expression of CXCL2 gene in the SVEC4-10 model of lymphatic endothelial-like cells which recapitulate the in vivo preference for basal to apical directionality as happens during neutrophil movement into medullary sinuses (Xiong et al., 2017). Similar to results in bone marrow cells, the levels of CXCL2 mRNA were increased by postprandial TRLs in SVEC4-10 cells and this enhancement was particularly pronounced by the postprandial TRL-SFAs (Fig. 4**B**). The effect of postprandial TRL MUFAs + EPA + DHA was less potent than that observed by the postprandial TRL-MUFAs (Fig. 4B). Coincidentally, the mRNA expression of CXCR2 also increased in a fatty-acid-dependent manner in bone marrow neutrophils at postprandial (OFL-SFAs > OFL-MUFAs = OFL-MUFAs + EPA + DHA) (Fig. 4**C**). At fasting, the increased levels of CXCR2 mRNA in bone marrow neutrophils were independent of the type of HFD and less strong than at postprandial (Fig. 4C). Next, the serum levels of CXCL2 at fasting and at postprandial were measured. We found that only the HFD-SFAs, but not the HFD-MUFAs and HFD-MUFAs + EPA + DHA, sharply increased the serum level of CXCL2 at fasting in our mice (Fig. 4**D**). It is worthy of notice that postprandial serum level of CXCL2 after administration of the OFL-SFAs was higher than that observed at fasting (Fig. 4D). Moreover, an increase of postprandial serum level of CXCL2 emerged after administration of the OFL-MUFAs, but to a lower extent than after the OFL-SFAs. Therefore, we also investigated if postprandial TRLs isolated from healthy volunteers that ingested meals enriched in SFAs, MUFAs or MUFAs + EPA + DHA could directly affect neutrophil migration using a robust ex vivo model with SVEC4-10 cells seeded on transwell inserts and freshly isolated murine bone marrow neutrophils placed in the top of chamber. None of postprandial TRLs, added to the upper or lower chamber, had any effect per se on bone marrow neutrophil migration (data not shown). However, postprandial TRLs when added after CXCL2 was in the bottom compartment had contrasting effects, with the TRL-SFAs favouring and the TRLs enriched in MUFAs (TRL-MUFAs ≈ TRL-MUFAs + EPA + DHA) obstructing the CXCL2-induced bone marrow neutrophil chemotaxis (Fig. 4**E**). The addition of an inhibitor of CXCR2 not only voided the CXCL2-mediated guiding of bone marrow neutrophils but also the positive influence of TRL-SFAs on this response.

CXCL12 is another prominent chemokine but involved in the retention of neutrophils in the bone marrow; constitutively expressed by bone marrow stromal cells, CXCL12 is the single natural ligand for CXCR4 receptor (De Filippo & Rankin, 2018). Therefore, we next sought to investigate the effects of HFDs at fasting and OFLs at postprandial on this axis by analysing the gene expression of CXCL12 in bone marrow cells and the surface expression of CXCR4 in bone marrow neutrophils. The HFD-MUFAs and HFD-MUFAs + EPA + DHA were observed to reduce at fasting the levels of CXCL12 mRNA in bone marrow cells when compared to the LFD and HFD-SFAs (Fig. 5**A**). This effect was also observed at postprandial with all of the OFLs according to the following pattern: OFL-SFAs > OFL-MUFAs = OFL-MUFAs + EPA + DHA (Fig. 5A). On the other hand, the FACS analysis for CXCR4 in bone marrow neutrophils showed that this receptor was only affected by OFLs, decreasing in the surface of bone marrow neutrophils according to a pattern that mirrored the effects of OFLs on gene expression of CXCL12 in bone marrow cells (Fig. 5**B**). Therefore, it is remarkable that CXCL12-CXCR4 and CXCL2-CXCR2 axes were oppositely regulated by OFLs.

3.6. Influence of HFMs on circulating neutrophil numbers in healthy volunteers

To translate the postprandial results to humans, we examined whether the postprandial neutrophilia induced by dietary fatty acids in the animal model could be observable in human subjects receiving HFMs. After 1-week pre-challenge feeding period on planned menus with the inclusion of dietary SFAs, MUFAs or MUFAs + EPA + DHA to assess baseline states, HFM-SFAs, HFM-MUFAs or HFM-MUFAs + EPA + DHA were administered to male healthy volunteers fasted for 12 h. The participants also received an OCM (meal with no-fat). At 2-h postprandial, the values of plasma TGs increased up to 139.4 ± 42.1 mg/dL after the OFM-SFAs, 123.0 ± 43.3 mg/dL after the OFM-MUFAs and 81.4 ± 34.2 mg/dL after the OFM-MUFAs + EPA + DHA. These values did not statistically differ among each other. As expected, the plasma TG concentration was postprandially unchanged (5.4 ± 6.0 mg/dL, mean value over a 6-h postprandial period) by the OCM. Despite OFMs induced comparable postprandial hypertriglyceridemic responses in the participants, they experienced increased number of circulating neutrophils depending on the OFM ingested (Fig. 6**A**). As similarly shown in mice, a more pronounced effect was observed with the OFM-SFAs, the effects of OFM-MUFAs and OFM-MUFAs + EPA + DHA were analogous, and the postprandial levels of circulating neutrophils did not correlate with postprandial levels of circulating TGs ($r = 0.7711$; $p = 0.4394$) in the participants. Furthermore, the OFMs increased the levels of MMP9 mRNA in circulating neutrophils (Fig. 6**B**) and of MMP9 protein release in plasma (Fig. 6**C**) in a parallel fashion (HFM-SFAs > HFM-MUFAs = HFM-MUFAs + EPA + DHA) as circulating neutrophils did. After only 1-week of planned menus containing dietary SFAs, MMP9 in plasma was also increased at fasting (Fig. 6C).

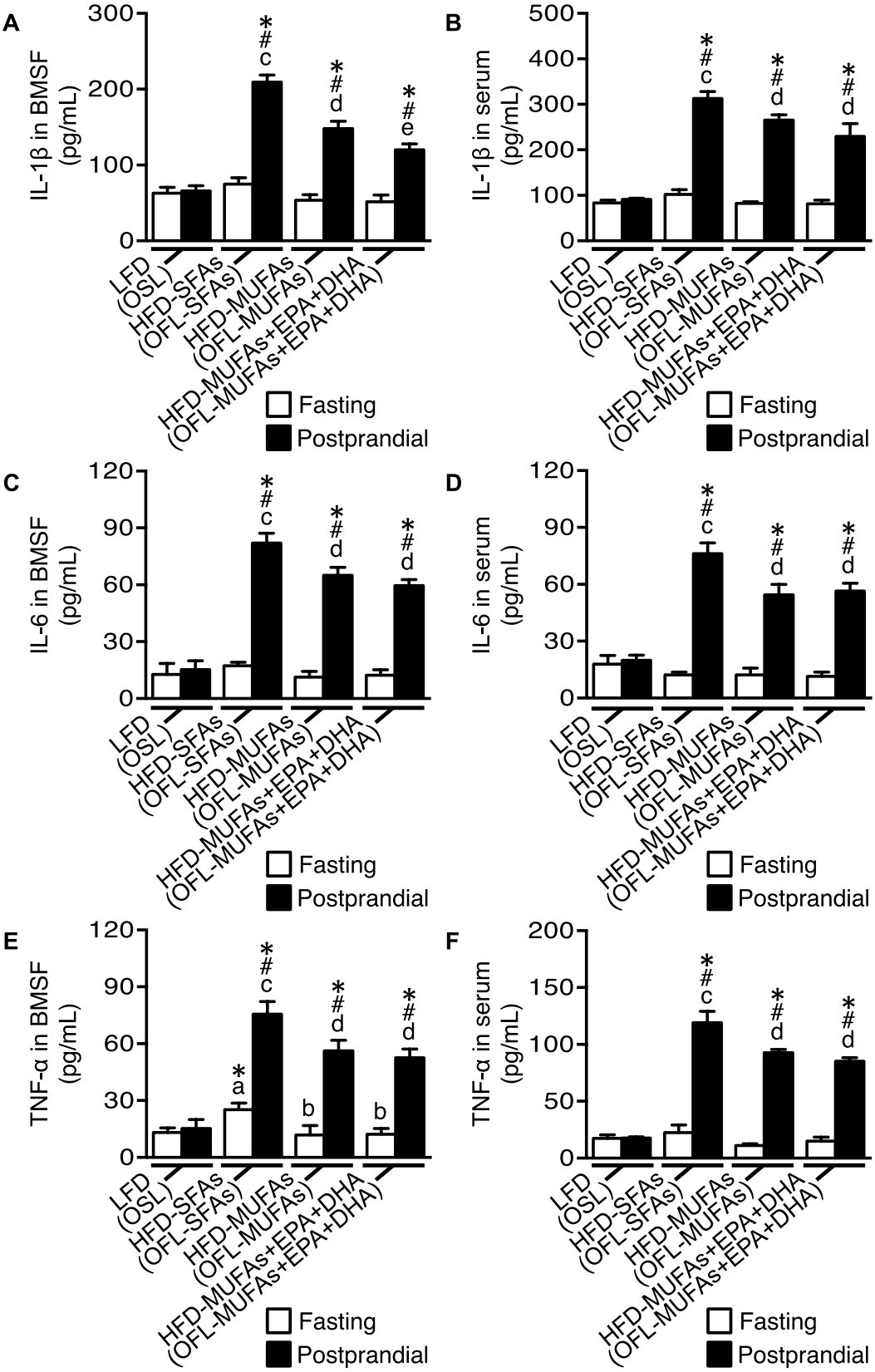

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Fig. 3. The type of dietary fat in high-fat diets (HFDs) and oral-fat loads (OFLs) modulates the presence of IL-1β, IL-6 and TNF-α in bone marrow supernatant fluid (BMSF) and serum of C57BL/6J mice. Animals were fed a low-fat diet (LFD) or HFDs containing anhydrous butter (HFD-SFAs), olive oil (HFD-MUFAs) or olive oil plus EPA + DHA (HFD-MUFAs + EPA + DHA) for 4 weeks. Serum and BMSF were obtained at fasting (at the end of the diet period, $n = 9$) and at postprandial (after the administration of the oral loads, $n = 9$). (**A**) Levels of IL-1β in BMSF at fasting (white) and at postprandial after the animals received an oral-saline load (OSL) or an OFL of melted anhydrous butter (OFL-SFAs), olive oil (OFL-MUFAs) or olive oil plus EPA + DHA (OFL-MUFAs + EPA + DHA) (black). (**B**) Levels of IL-1β in the serum at fasting (white) and at postprandial (black). (**C**) Levels of IL-6 in BMSF at fasting (white) and at postprandial (black). (**D**) Levels of IL-6 in the serum at fasting (white) and at postprandial (black). (**E**) Levels of TNF-α in BMSF at fasting (white) and at postprandial (black). (**F**) Levels of TNF-α in the serum at fasting (white) and at postprandial (black). For all graphs, the bar heights reflect the mean and the error bars represent *SD* of nine independent biological replicates. *Significant difference of HFD or OFL compared to LFD or OSL. #Significant difference of postprandial value compared to fasting value within each dietary fat. Different letters assigned to fasting values (a and b) or to postprandial values (c, d and e) indicate significant differences between HFDs or OFLs.

4. Discussion

The aim of this study was to analyse the impact of dietary SFAs, MUFAs and MUFAs supplemented with EPA and DHA at fasting (when administered in HFDs for a short-term period of 4 weeks) and at postprandial (when administered in OFLs by gavage) on the mobilization of the neutrophils between bone marrow and blood in wild-type male C57BL/6J mice. The impact of the same type of dietary fats in OFMs when ingested by healthy men volunteers on postprandial circulating neutrophil counts was also assessed to translate findings based on mouse research into clinical settings. Our findings, which should not be extended to women or female mice, reveal that those dietary fatty acids act as critical regulators of rapid neutrophil trafficking from bone marrow. Of importance, this capacity occurs both in mice and humans and operates in a manner that depends on the predominant type of fatty acids in the dietary fat ingested. The most potent dietary fat for eliciting robust bone marrow neutrophil mobilization was that enriched in SFAs. In contrast, dietary fats enriched in MUFAs or in MUFAs supplemented with EPA and DHA had mild or even no effects. These findings enlarge our previous knowledge on the role of different dietary fats in the lipid-accumulation and activation of bone marrow neutrophils (Ortega-Gomez et al., 2017) and of HFD-driven induction of hypercholesterolemia in the increased number of circulating neutrophils and early atherosclerotic lesions (Drechsler, Megens, van Zandvoort, Weber, & Soehnlein, 2010) in Apoe$^{-/-}$ mice. Therefore, this is further evidence that exogenous fatty acids, both directly or indirectly, can rapidly and sensitively reprogram the metabolic footprint of neutrophils and their environment in the bone marrow toward a migratory phenotype.

While bone marrow neutrophils were increased at fasting only in wild-type C57BL/6J mice fed with the HFD-SFAs for 4 weeks, previous studies reported that the frequency of bone marrow neutrophils is slightly increased with the HFD-MUFAs or the HFD-MUFAs-EPA + DHA and decreased with the HFD-SFAs at fasting in the same strain of mice when the feeding period was extended to 20 weeks, that is when animals became obese (Lemus-Conejo et al., 2021). One explanation for this apparent paradox may relate to the different impact of HFDs and their fatty acids on white adipose tissue expansion and bone marrow at the onset of obesity and at the well-established obesity (Bowers & Singer, 2021, Lemus-Conejo et al., 2021, Montserrat-de la Paz et al., 2019). In the short-term exposure to the HFD-SFAs (4 weeks), we observed the induction of apoptosis in bone marrow neutrophils, which could be probably due to an excessive engulfment of TGs (Ortega-Gomez et al., 2017) and not to the trafficking of senescent neutrophils back to the bone marrow, as CXCR4 expression in circulating and bone marrow neutrophils and CXCL12 expression in bone marrow cells at fasting were unaffected (De Filippo & Rankin, 2018). In addition, we found that the HFD-SFAs induced a remarkable elevation of fasting serum CXCL2, which is a chemokine associated with the onset of inflammation and recently identified to cause early senescence and a functional decline of stem cells from bone marrow mesenchymal niche during the development of obesity in rats (Bi et al., 2021). These findings are in line with previous in vitro reports showing that palmitic acid was involved in mediating apoptosis and pro-inflammatory response in immune cells, which were effects not observed with oleic acid, EPA or DHA (Hidalgo, Carretta, & Burgos, 2021, Radzikowska et al., 2019). It is known that the sterile inflammation in the bone marrow microenvironment can converge in the activation of granulocytes (Markovic, Maslovaric, Djikic, & Cokic, 2022) and that the feeding of a lard-enriched HFD for only 1 week to wild-type C57BL/6J mice can enhance differentiation of myeloid progenitors into neutrophils (Huang et al., 2017). Therefore, our data support the notion that the occurrence of early apoptotic events in bone marrow neutrophils during the early-phase of obesity can be compensated with increased production, activation and mobilisation of new neutrophils from the own bone marrow. This could happen by sensing dying signals (Rosales, 2018). We further demonstrate that the replacement of dietary SFAs with MUFAs or MUFAs plus EPA and DHA in HFDs largely prevents bone marrow neutrophil stress and exaggerated neutrophil exit from and return to bone marrow niches. As shown previously, the obese phenotype of mice after long-term exposure to HFDs (20 weeks) is distinguished by a lower weight gain and visceral adipose inflammation if the HFD is enriched in MUFAs (without or with added EPA and DHA) instead of SFAs (Lemus-Conejo et al., 2021). Nonetheless, these obese animals exhibited similar bone marrow neutrophil viability irrespective of the type of HFD, which evidence the end result of a homeostatic regulation of the bone marrow neutrophil compartment when obesity has already been established. In this ongoing process, our study provides novel evidence that dietary MUFAs are effector molecules able to obstruct the involutional bone marrow neutrophil loss seen in mice exposed to dietary SFAs during HFD, resembling trained immunity conditions (Netea et al., 2020).

At postprandial, the OFLs did not induce apparent neutrophil production in the bone marrow of mice but rather neutrophil mobilisation from bone marrow to circulation, with blood neutrophil numbers even higher than those found in blood fasting samples from the same mice after the exposure to the HFDs for 4 weeks. These findings suggest an extreme sensitivity of bone marrow to overcome the retention of neutrophils in response to dietary fatty acids during the postprandial state. The OFL-SFAs elicited more evident effects and the supplementation of the OFL-MUFAs with EPA and DHA reduced the potential of the OFL-MUFAs to mobilize neutrophils from bone marrow. To our knowledge, no previous studies have addressed the acute impact of different types of dietary fatty acids in OFLs on bone marrow neutrophil release. Postprandial excursions of TGs also include transient systemic inflammation (Mazidi et al., 2021). Therefore, it is likely that neutrophil exit from bone marrow niches could be at least in part a response against these postprandial inflammatory signals. Our study also shows to what extent different dietary fatty acids (SFAs > MUFAs) influence bone marrow neutrophil apoptosis at postprandial (in vivo) and through the involvement of postprandial TRLs (in vitro). Furthermore, in parallel to the increased bone marrow neutrophil mobilisation in mice submitted to OFLs, the apoptosis levels of bone marrow neutrophils at postprandial were higher than those observed at fasting. Accordingly, the pro-inflammatory triumvirate of IL-6, IL-1β and TNF-α were increased in BMSF at postprandial, particularly in mice submitted to the OFL-SFAs. The higher engulfment of TGs from postprandial TRL-SFAs than from postprandial TRL-MUFAs by bone marrow neutrophils of Apoe$^{-/-}$ mice (Ortega-Gomez et al., 2017) could explain the apoptotic data in our mice. However, the relative contribution of extramedullary and intramedullary programs of inflammation, due or not due to bone marrow neutrophil apoptosis, in the traffic of neutrophils from bone marrow to

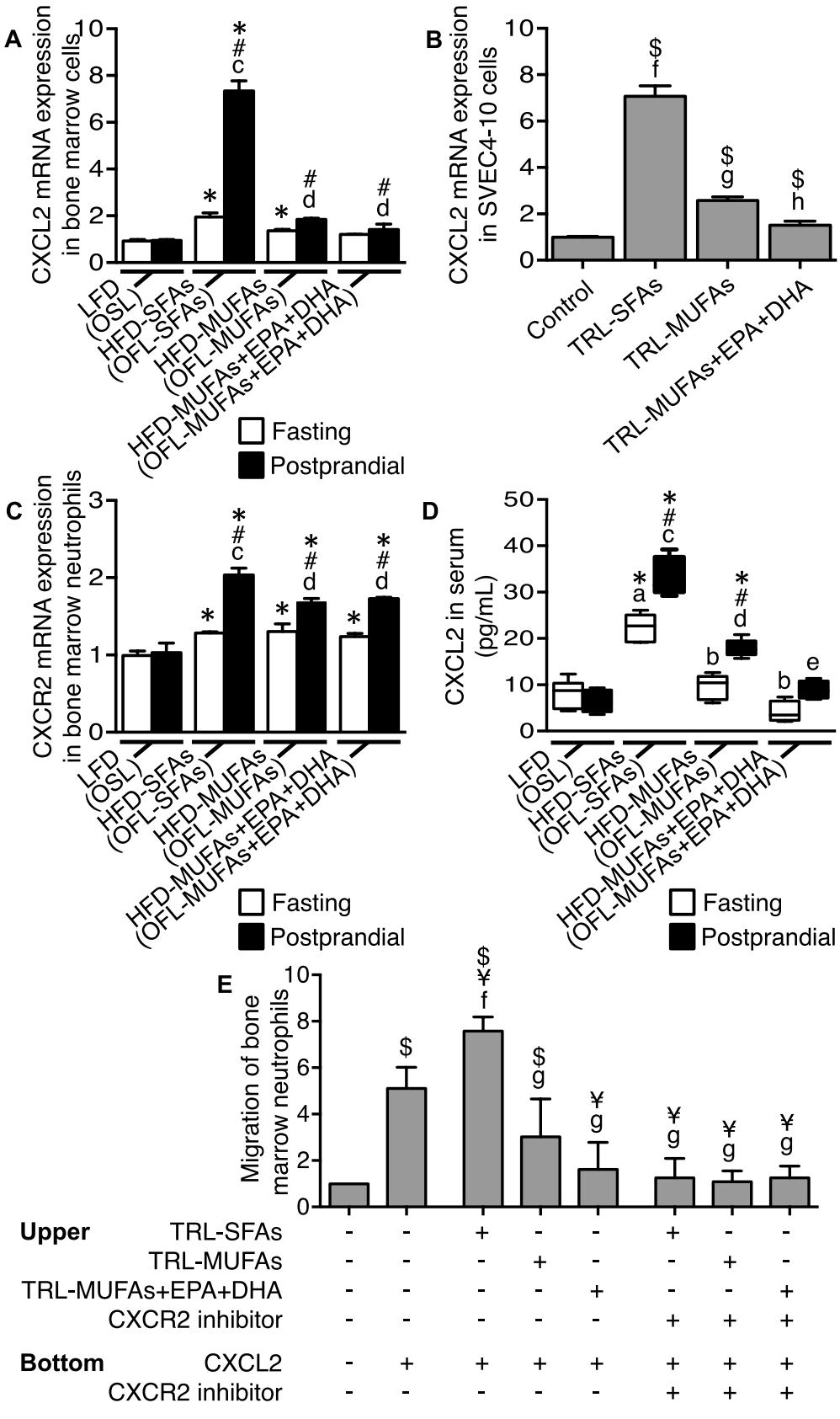

Fig. 4. The type of dietary fat in high-fat diets (HFDs), oral-fat loads (OFLs) and postprandial triglyceride-rich lipoproteins (TRLs) modulates the bone marrow neutrophil CXCL2-CXCR2 axis and migration of C57BL/6J mice. Animals were fed a low-fat diet (LFD) or HFDs containing anhydrous butter (HFD-SFAs), olive oil (HFD-MUFAs) or olive oil plus EPA + DHA (HFD-MUFAs + EPA + DHA) for 4 weeks. Serum and bone marrow were obtained at fasting (at the end of the diet period, $n = 9$) and at postprandial (after the administration of the oral loads, $n = 9$). (**A**) Relative expression of CXCL2 gene in bone marrow cells at fasting (white), and at postprandial after the animals received an oral-saline load (OSL) or an OFL of melted anhydrous butter (OFL-SFAs), olive oil (OFL-MUFAs) or olive oil plus EPA + DHA (OFL-MUFAs + EPA + DHA) (black). (**B**) Relative expression of CXCL2 gene in SVEC4-10 cells after incubation with human postprandial TRLs (100 μg of TGs/mL) for 12 h. The different TRLs were isolated from postprandial serum samples of healthy volunteers after the intake of oral-fat meals containing anhydrous butter (TRL-SFAs), olive oil (TRL-MUFAs) or olive oil plus a dose of EPA + DHA (TRL-MUFAs + EPA + DHA). (**C**) Relative expression of CXCR2 in bone marrow neutrophils at fasting (white) and at postprandial (black). (**D**) Levels of CXCL2 in the serum of mice at fasting (white) and at postprandial (black). (**E**) Bone marrow neutrophil migration across SVEC4-10 cells with postprandial TRLs, CXCL2 (100 ng/mL) and a CXCR2 inhibitor (200 nM) as indicated. For all graphs, the bar heights reflect the mean and the error bars represent *SD* of nine independent biological replicates. *Significant difference of HFD or OFL compared to LFD or OSL. #Significant difference of postprandial value compared to fasting value within each dietary fat. Different letters assigned to fasting values (a and b) or to postprandial values (c, d and e) indicate significant differences between HFDs or OFLs. $Significant difference of the treatment compared to untreated cells. ¥Significant difference of the treatment with postprandial TRLs compared to CXCL2-treated cells. Different letters (f, g and h) indicate significant differences between postprandial TRLs.

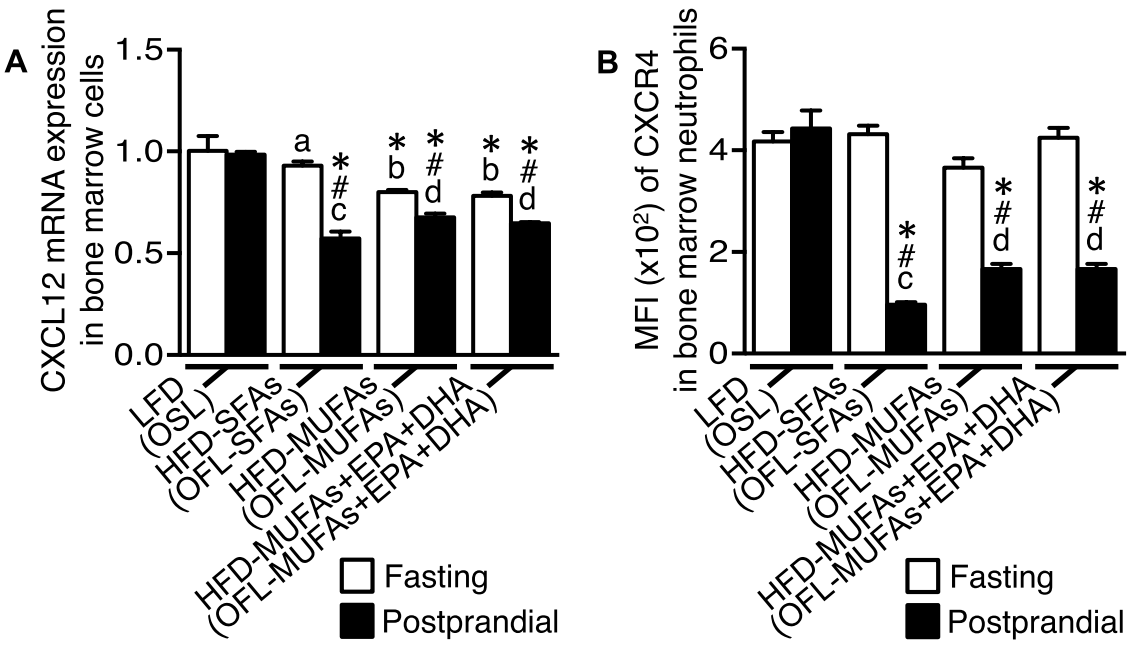

Fig. 5. The type of dietary fat in high-fat diets (HFDs) and oral-fat loads (OFLs) modulates the bone marrow neutrophil CXCL12-CXCR4 axis of C57BL/6J mice. Animals were fed a low-fat diet (LFD) or HFDs containing anhydrous butter (HFD-SFAs), olive oil (HFD-MUFAs) or olive oil plus EPA + DHA (HFD-MUFAs + EPA + DHA) for 4 weeks. Bone marrow was obtained at fasting (at the end of the diet period, $n = 9$) and at postprandial (after the administration of the oral loads, $n = 9$). (**A**) Relative expression of CXCL12 gene in bone marrow cells at fasting (white), and at postprandial after the animals received an oral-saline load (OSL) or an OFL of melted anhydrous butter (OFL-SFAs), olive oil (OFL-MUFAs) or olive oil plus EPA + DHA (OFL-MUFAs + EPA + DHA) (black). (**B**) Mean fluorescence intensity (MFI) of CXCR4 in bone marrow neutrophils at fasting (white) and at postprandial (black). For all graphs, the bar heights reflect the mean and the error bars represent *SD* of nine independent biological replicates. *Significant difference of HFD or OFL compared to LFD or OSL. #Significant difference of postprandial value compared to fasting value within each dietary fat. Different letters assigned to fasting values (a and b) or to postprandial values (c and d) indicate significant differences between HFDs or OFLs.

blood at postprandial remains to be clarified. An intriguing observation was that the bone marrow neutrophil pool was not homeostatically replenished during the postprandial state after the administration of the OFL-SFAs and OFL-MUFAs, which may suggest a transient suppression of granulopoiesis or delay in the arrival of new stocks of neutrophils. These topics, particularly for how long could be "the window" with bone marrow depleted of neutrophils, are worth to investigate more comprehensively in future studies.

The mechanisms through which dietary fatty acids in HFDs for a short-term period of 4 weeks or OFLs by gavage influence the navigation of neutrophils between bone marrow and blood are largely unknown. The CXCL2/CXCR2 and CXCL12/CXCR4 chemokine axes are key regulators of neutrophil trafficking in the bone marrow (De Filippo & Rankin, 2018, Eash et al., 2010, Sawant et al., 2021). We found that the HFD-SFAs, but not the HFDs enriched in MUFAs, dramatically increased the levels of CXCL2 in serum. This increase was even more marked in postprandial serum samples. It is the first time, to our knowledge, that dietary SFAs are reported to enhance the release of a neutrophil-mobilizing agent into the circulation. In addition, the levels of CXCL2 mRNA in bone marrow cells and of CXCR2 mRNA in bone marrow neutrophils were slightly and similarly increased by all the HFDs, which can stem from high-fat overfeeding and not from the specificity of dietary fatty acids in HFDs. This was not the case at postprandial, as the effects of OFL-MUFAs and OFL-MUFAs + EPA + DHA on the transcriptional activity of CXCL2 and CXCR2 genes were lower than those of OFL-SFAs, which may be indicative that mechanisms operating to modulate the expression of these genes in the bone marrow are fatty-acid-sensitive only in the acute period of feeding. Another interesting finding was the upregulation of CXCL2 gene in endothelial SVEC4-10 cells by postprandial TRLs, with the TRL-SFAs exerting the maximum effect. The production of CXCL2 by neutrophils is implicated in neutrophil breaching of endothelial cell junctions (Girbl et al., 2018). In this line, elevated CXCL2 levels concurring with the rise of neutrophils in the circulation and the inflammation of coronary artery endothelial cells in response to dietary SFAs were reported to be associated with pathological disorders that affect the endothelial vasculature (Krogmann et al., 2011), and may be indicative of a cue by way of dietary SFAs promote the navigation of neutrophils from bone marrow to blood, for which the passage of neutrophils through the sinusoidal endothelium is needed (Kraus & Gruber, 2021). Importantly, the lower or no effects of dietary MUFAs in this system and the recent association of oleic acid alone (produced through the catalysis of a functional genetic variation of stearoyl-CoA desaturase) or in combination with omega-3 PUFA supplementation in determining a decreased risk of coronary artery disease in humans (Liu et al., 2020) points to a potential selective impact of dietary fatty acids on the bone marrow endothelial vasculature. We explored this in the SVEC4-10 model of lymphatic endothelial-like cells to recapitulate the platform where trafficking into and out of the bone marrow occurs (Xiong et al., 2017), and we observed that the TRL-SFAs enhanced CXCL2-induced transmigration of bone marrow neutrophils, while the TRLs enriched in MUFAs, particularly the TRL-MUFAs + EPA + DHA, had opposite effects. These findings, together with those of the CXCR2 inhibitor abolishing any transendothelial trafficking, suggest a robust role for the CXCL2-CXCR2 axis in the mobilization of bone marrow neutrophils by dietary fatty acids. The search of new bone marrow mobilization strategies through the activation of CXCR2 and the deactivation or depletion of CXCR4 has confirmed that CXCR2 can modulate bone marrow neutrophil mobilization when CXCR4 signalling is inhibited (Eash et al., 2010) and has documented that this mechanism, including the increase of bone marrow vascular permeability, may be governed by MMP9, resulting in enhanced MMP9 release in plasma (Kapellos et al., 2019). MMP9 is produced during maturation of neutrophils in the bone marrow and plays a crucial role in the activation of CXCR2 on neutrophils (Rawat, Syeda, & Shrivastava, 2021). Interestingly, we found that the expression of CXCR4 in bone marrow neutrophils was repressed by OFLs. The most potent effect was shown by the

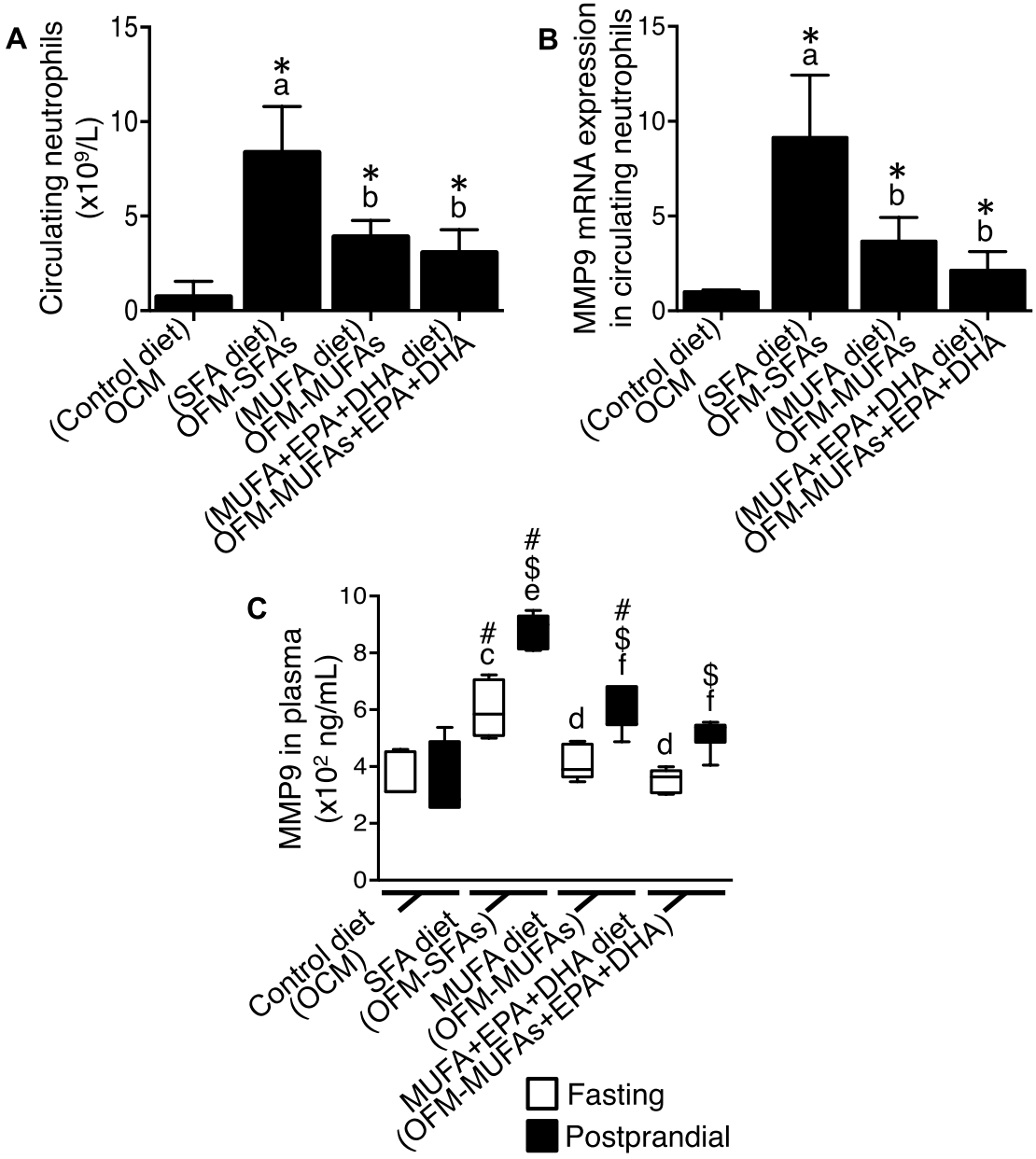

Fig. 6. The type of dietary fat in planned diets for 1 week and in high-fat meals (HFMs) modulates the number of neutrophils in the blood of healthy volunteers. Twelve participants were provided 1 week of planned menus with the inclusion of anhydrous butter (SFA diet), olive oil (MUFA diet) or olive oil plus EPA + DHA (MUFA + EPA + DHA diet). Blood (and plasma) was obtained at fasting (at the end of the pre-challenge period) and at postprandial (after the administration of the meals). (**A**) Number of circulating neutrophils at postprandial after the participants ingested an oral-control meal (OCM) with no fat or an OFM containing anhydrous butter (OFM-SFAs), olive oil (OFM-MUFAs) or olive oil plus EPA + DHA (OFM-MUFAs + EPA + DHA). (**B**) Relative expression of MMP9 gene in circulating neutrophils at postprandial. (**C**) Levels of MMP9 in plasma at fasting (white) and at postprandial (black). For all graphs, the bar heights reflect the mean and the error bars represent *SD* of twelve independent biological replicates. *Significant difference of OFMs compared to OCM. Different letters (a and b) indicate significant differences between OFMs. #Significant difference of diets enriched in SFAs, MUFAs or MUFAs + EPA + DHA for 1 week period or OFMs compared to control diet or OCM. $Significant difference of postprandial value compared to fasting value within each dietary fat. Different letters assigned to fasting values (c and d) or to postprandial values (e and f) indicate significant differences between diets enriched in SFAs, MUFAs or MUFAs + EPA + DHA for 1 week period or between OFMs.

OFL-SFAs. Hence, our data support a selective role for dietary fatty acids in the attenuation of CXCR4 pathways and thereby in the retention signals for neutrophils in the bone marrow, which, in turn, may favour the rapid enrichment in neutrophils of the blood compartment during the postprandial state. These observations were validated in healthy volunteers showing that the OFM-SFAs was more efficient than OFMs enriched in MUFAs to induce an increase of circulating neutrophils at postprandial. Our study further suggests the potential involvement of MMP9 in the neutrophil navigation of healthy volunteers, as the levels of MMP9 mRNA in circulating neutrophils and MMP9 in plasma increased in parallel with the postprandial neutrophilia.

5. Conclusions

In summary, the results taken together place dietary fatty acids as novel and selective modulators of neutrophil behaviour in the bone marrow early in the course of high-fat diets even after the intake of high-fat meals. In contrast to the effects of dietary SFAs in promotion bone

marrow neutrophil apoptosis, inflammation and the neutrophil traffic from bone marrow to blood via the CXCL2-CXCR2 axis, MUFAs induce favourable survival, inflammation and immune signatures, as well as favourable lipid profile. Additional studies should be needed to more clearly delineate the independent and rapid effects of dietary SFAs and MUFAs on factors governing the viability and functionality of bone marrow neutrophils, and the active migration of these neutrophils from bone marrow niches. This original information may be valuable to establish the clinical relevance of dietary fatty acid-based strategies that could help delay the onset of obesity-associated health risks.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported by MCIN/AEI/10.13039/501100011033 and by "ERDF A way of making Europe" (grant number AGL2016-80852-R). Sergio Lopez and Lourdes M. Varela acknowledge financial support from the "V Own Research Plan" of the University of Seville (VPPI-US) cofounded by the European Social Fund. We thank the Core Facilities for Oil Extraction and Refining and the Cell Biology Unit at the Instituto de la Grasa (CSIC, Seville) and the Mouse Facility at the CABD (UPO/JA/CSIC, Seville) for their assistance during the fulfilment of this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochms.2022.100133.

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