Scope: The role of dietary fatty acids in the generation of bone marrow (BM) immune cells and their trafficking to extramedullary compartments in the obesity is not yet fully understood.

Methods and Results: C57BL/6J mice are randomly assigned to isocaloric high-fat diets (HFDs) formulate with dietary fats rich in saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) or MUFAs fortified with eicosapentaenoic and docosahexaenoic acids for 20 weeks, followed by profiling of the obese metabolic phenotype and immunophenotypic features of immune cells in blood, spleen, and BM. All HFDs induce an obese phenotype, but it becomes largely less disruptive after the HFDs are enriched in MUFAs, which also induce signs of granulopoiesis and an expansion of long-term hematopoietic stem and granulocyte-macrophage progenitor cells in BM. In contrast, a HFD enriched in SFAs disturbs the fitness of medullary lymphocytes and promotes monopoiesis in favor of pro-inflammatory activated subsets.

Conclusion: The reshaping of the fatty acid pools with MUFAs from the diet serves to manipulate the generation and trafficking of immune cells that are biased during obesity. These findings reveal a novel strategy by which dietary MUFAs may be instrumental in combating HFD-induced dysfunctional immune systems.

1. Introduction

The increase in weight and adipose tissue is known as a main driver of overweight and obesity, which result from societal factors, among the most common being the consumption of high-fat diets (HFDs) that are typically rich in saturated fatty acids (SFAs). The high risk of accompanying cardiovascular comorbid disorders remains a major concern for patients who suffer from overweight or obesity. In a provocative way, unlike focusing on the quantitative reduction in energy intake to induce weight loss, growing evidence supports that qualitative changes without energy restriction in a HFD may be an additional choice in modern times in which high-fat foods are readily available and accessible and are preferred foods in many communities. An illustrative example is the European randomized controlled trial, the PREDIMED (Prevención con Dieta Mediterránea) study, showing that a dietary pattern with an intake that reaches and even exceeds 40% of energy from fats can decrease the occurrence of myocardial infarction, stroke, and cardiovascular death. In this trial, the Mediterranean-style diet was supplemented with additional olive oil as the main source of fat, which agrees with the potential relevance of the monounsaturated fatty acid (MUFA) oleic acid to prevent the development of cardiovascular risk and to manage obesity, as shown in a recent systematic review. Moreover, long-chain ω-3 polyunsaturated fatty acids (PUFAs), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been...
Cardiovascular complications due to inflammation and cellular immunity abnormalities in obesity are different sides of the same coin. Obesity is indeed linked with changes in the bone marrow (BM), leading to imperfect innate and adaptive immune responses. In BM, immune cells arise from hematopoietic stem cells (HSCs), giving rise to hematopoiesis in all blood cell types. Marginal pools of immune cells capable of mediating innate and adaptive immunity can also be found in extramedullary tissues such as the spleen.

The homeostasis of HSCs in the BM niche is essential for normal long-term hematopoiesis and the regular abundance of selective mature hematopoietic populations in blood and spleen, the disruption of which may lead to loss of health. An important overlooked point is that most of the HFDs that have been used to model the immune system in fed mice are formulated with lard is often confused as being high in saturated fat, but it is not. Lard is composed of oleic acid (18:1) in inflammatory activated subsets. Phocytes in the BM and promoted monopoiesis in favor of pro-

2. Results

2.1. Effects of Fatty Acids in HFDs on the Obese Phenotype

As shown in Table S2, Supporting Information, the principal fatty acid in HFD-SFAs was palmitic acid (16:0, >30% of total fatty acids), and the percentage of total SFAs was close to 60%. HFD-MUFA and HFD-MUFA+EPA+DHA were primarily composed of oleic acid (18:1o-9, by ≈70%), but the latter also contained 1.3% EPA (20:5o-3) and 1% DHA (22:6o-3). We found no changes in feeding habits, with similar food and water intakes in mice on either the low-fat diet (LFD) or HFDs (data not shown). Compared to the LFD, all of the HFDs induced an increase (p < 0.001 vs LFD) in body mass, but this was higher (p < 0.001) after HFD-SFAs (+81%) than after HFD-MUFA (+31%) and HFD-MUFA+EPA+DHA (+24%) at the end of the 20-week dietary regimen (Table S5, Supporting Information). In a similar fashion, the mass of eWAT, which is predictive of obesity-related co-morbidities [26] and low bone mass, [27] was increased in mice upon exposure to HFDs, and it was higher after HFD-SFAs than after HFD-MUFA and HFD-MUFA+EPA+DHA (Table S5, Supporting Information).

Mice fed HFDs also developed dyslipidaemia characterized by an increase in the levels of total cholesterol and triglycerides, with HFD-SFAs again exerting the strongest effects (Table S5, Supporting Information). In eWAT, we detected the mRNA expression of genes involved in inflammation (interleukin 6 [Il6] in the infiltration of monocyte-derived macrophages [28]; neutrophil elastase [Elane] as a marker of neutrophil recruitment [29], and CCAAT/enhancer-binding protein beta [Cebpb] as a regulator of adipocyte differentiation but also of dietary fatty acid-mediated inflammation [30] in adipose depots), adipogenesis (peroxisome proliferator-activated receptor gamma [Pparg] [31]) and thermogenesis (uncoupling protein 1 [Ucp1] and iodothyronine deiodinase 2 [Dio2] as markers of “beige” adipocytes [32,33]) by real-time PCR. Mice fed HFDs had increased expression levels of the Il6 gene, but these levels were lower after feeding HFDs enriched in MUFA (Figure 1A). The Elane gene was dramatically repressed after HFDs enriched in MUFA (Figure 1B). The Cebpb gene was also upregulated after HFD-SFAs but downregulated after HFDs enriched in MUFA compared to the LFD (Figure 1C). Mice fed HFDs had decreased expression levels of the Pparg (Figure 1D) and Ucp1 (Figure 1E) genes, which were lower after HFD-SFAs than after HFDs enriched in MUFA. The Dio2 gene was also downregulated after HFD-SFAs, but its expression remained unchanged after HFDs were enriched in MUFA (Figure 1F). No significant differences were observed between HFD-MUFA and HFD-MUFA+EPA+DHA. Therefore, long-term administration of HFDs may induce obesity, adipose inflammation, and dyslipidaemia in wild-type C57BL/6j mice; however, this obese phenotype is characterized by significant, minor metabolic perturbations if the HFD is enriched in MUFA in place of SFAs.

2.2. Effects of Fatty Acids in HFDs on the Principal Composition of Free Fatty Acids in BM Extracellular Fluid

As shown in Figure S2, Supporting Information, total MUFA were increased in BM extracellular fluid of mice on the HFD-MUFA and HFD-MUFA+EPA+DHA, with no differences between these groups, while the proportion of total SFAs and PUFA remained similar after any diet. The ratios of palmitic acid to oleic acid and SFAs to MUFA in this compartment were increased in BM extracellular fluid of mice on the HFD-SFAs or LFD. EPA and DHA were not detected. Details of the protocol can be found in the Supplemental Material.

2.3. Effects of Fatty Acids in HFDs on Mature Hematopoietic Lineage Cells

A panel of antibodies and the gating strategy illustrated in Figure 2 were used for immunophenotyping blood lymphoid and myeloid subsets by flow cytometry. It was first...
Figure 1. High-fat diets enriched in SFAs (anhydrous butter, HFD-SFAs), MUFAs (olive oil, HFD-MUFAs), or MUFAs (olive oil) fortified with EPA and DHA (HFD-MUFAs+EPA+DHA) on obese phenotype in wild-type C57BL/6J mice. Reverse transcription-quantitative PCR analysis of key hub genes (A) \textit{Il6}, (B) \textit{Elane}, and (C) \textit{Cebpb} involved in inflammation, (D) \textit{Pparg} involved in adipogenesis, (E) \textit{Ucp1}, and (F) \textit{Dio2} involved in thermogenesis in epididymal white adipose tissue (eWAT) at the end of dietary regimens. Values are individual data points ±SD; n = 20/group. Differences between groups were assessed by two-way ANOVA with Tukey’s post hoc test. ***, \( p < 0.001 \); **, \( p < 0.01 \); *, \( p < 0.05 \) (compared to all other study groups and as indicated).

Demonstrated by blood count assays that HFDs enriched in MUFAs, with no difference between HFD-MUFAs and HFD-MUFAs+EPA+DHA, increased the numbers of white blood cells (Figure 3A) and neutrophils (Figure 3B), while the numbers of lymphocytes (Figure 3C) and monocytes (Figure 3D) remained unchanged in the peripheral blood of all four groups of animals. It was also found that HFDs increased the subpopulation of B cells (CD45\(^+\)B220\(^+\) cells) (Figure 3E) and that only HFD-SFAs reduced the subpopulation of T cells (CD45\(^+\)CD3\(^e\) cells) (Figure 3F). In addition, the fraction of Ly6C\(^{hi}\) monocytes (CD45\(^+\)B220 CD3\(^e\) CD11b\(^+\)Ly6G\(^-\)Ly6C\(^{hi}\) cells) was increased with HFD-SFAs but decreased with HFD-MUFAs+EPA+DHA (Figure 3G), and the fraction of Ly6C\(^{lo}\) monocytes (CD45\(^+\)B220 CD3\(^e\) CD11b\(^+\)Ly6G\(^-\)Ly6C\(^{lo}\) cells) remained unaffected (Figure 3H) by flow cytometry analysis. None of the HFD regimens induced significant changes compared with LFD on the numbers of red blood cells (Figure S3A, Supporting Information), hemoglobin (Figure S3B, Supporting Information), hematocrit (Figure S3C, Supporting Information), mean corpuscular volume (Figure S3D, Supporting Information), mean corpuscular hemoglobin (Figure S3E, Supporting Information), red cell distribution width (Figure S3F, Supporting Information), platelets (Figure S3G, Supporting Information), or mean platelet volume (Figure S3H, Supporting Information).

By differential expression of CD45 to identify the infiltration of BM cells into the spleen as a major extramedullary reservoir for storage and deployment of mature hematopoietic lineage cells\(^9\) and following a similar gating strategy to that used for blood (Figure S4, Supporting Information), no significant differences in live CD45\(^+\) cells in the spleen of mice fed HFDs were observed, but these values were lower than those in the spleen.
Figure 2. An example of the gating strategy used to identify B (CD45$^+$B220$^+$) and T (CD45$^+$CD3e$^+$) cells, and Ly6C$^\text{Hi}$ (CD45$^+$B220$^-$CD3e$^-$CD11b$^+$Ly6G$^-$Ly6C$^\text{Hi}$) and Ly6C$^\text{Lo}$ (CD45$^+$B220$^-$CD3e$^-$CD11b$^+$Ly6G$^-$Ly6C$^\text{Lo}$) monocytes in peripheral blood of wild-type C57BL/6J mice. CD45$^+$ viable cells were first individualized by crossing the singlet gate. CD, cluster of differentiation; FSC-A, forward scatter area; SSC-A, side scatter area.
Figure 3. High-fat diets enriched in SFAs (anhydrous butter, HFD-SFAs), MUFAs (olive oil, HFD-MUFAs), or MUFAs (olive oil) fortified with EPA and DHA (HFD-MUFAs+EPA+DHA) on mature hematopoietic lineage cells in peripheral blood of wild-type C57BL/6 mice. The number of (A) white blood cells, (B) neutrophils, (C) lymphocytes, and (D) monocytes by blood count assays, and the percentages of (E) B lymphocytes, (F) T lymphocytes, (G) Ly6CHi monocytes, and (H) Ly6Clo monocytes by flow cytometry at the end of 20-week dietary regimens. Viable cells (7-AAD–Annexin V–) were only considered. Values are individual data points ± SD; n = 20/group. Differences between groups were assessed by two-way ANOVA with Tukey’s post hoc test. ***, p < 0.001; **, p < 0.01; *, p < 0.05 (compared to all other study groups and as indicated).

of mice fed the LFD (Figure 4A). HFDs enriched in MUFAs increased splenic neutrophils (Figure 4B) and Ly6CHi monocytes (Figure 4C), while HFD-SFAs decreased splenic B cells (Figure 4D), and all HFDs decreased splenic T cells (Figure 4E) relative to CD45+ cells. This change in the cellular composition seen in the spleen of mice on HFD-SFAs was associated with increased levels of total cell death (+14%, p < 0.05). Furthermore, the spleen weights of mice on HFD-SFAs were higher than those of mice on the LFD or the HFDs enriched in MUFAs (Figure S5A, Supporting Information). The increase in spleen...
enlargement was previously shown to occur in mice after HFDs enriched in SFAs.\textsuperscript{[34]} However, when the spleen weight was normalized to body weight (Figure S5B, Supporting Information), the spleen-to-body-weight ratios remained the same for all four groups of animals (Figure S5C, Supporting Information).

These findings prompted us to further explore mature lymphoid and myeloid cells in BM. The gating strategy, similar to that used for blood and spleen, is shown in Figure S6, Supporting Information. It was first observed that the occurrence of total B and T cells (as a percentage of all BM cells) was similar among
High-fat diets enriched in SFAs (anhydrous butter, HFD-SFAs), MUFAs (olive oil, HFD-MUFAs) or MUFAs (olive oil) fortified with EPA and DHA (HFD-MUFAs+EPA+DHA) on mature hematopoietic lineage cells in bone marrow of wild-type C57BL/6J mice. The percentages of (A) live B lymphocytes, (B) dead B lymphocytes, (C) live T lymphocytes, (D) dead T lymphocytes, (E) neutrophils, (F) monocytes, (G) Ly6CHi, and (H) Ly6CLo monocyte subsets at the end of 20-week dietary regimens. Except where otherwise noted, viable cells (7-AAD–Annexin V–) were only considered. Dead cells were 7-AAD+. Values are individual data points ± SD; n = 20/group. Differences between groups were assessed by two-way ANOVA with Tukey’s post hoc test. ***: p < 0.001; **: p < 0.01; *: p < 0.05 (compared to all other study groups and as indicated).

the BM samples from any of the study groups. The frequency of live B cells in BM was not significantly different between mice after HFD-MUFAs or LFD; however, that found in the BM of mice after HFD-MUFAs+EPA+DHA and HFD-SFAs was increased and decreased, respectively (Figure 5A). HFD-SFAs also increased dead B cells in BM (Figure 5B). Likewise, the frequency of live (Figure 5C) and dead (Figure 5D) T cells was unaltered in the BM of mice after both HFDs enriched in MUFAs compared to the LFD, which was in contrast with the pattern of live (decreasing) and dead (increasing) T cells in the BM of mice after
HFD-SFAs. With respect to myeloid cells, the viability of neutrophils and monocytes in the BM was comparable in all groups (<8% of dead cells). HFD-MUFAs increased BM neutrophils, while HFD-SFAs induced the opposite effect (Figure 5E). Even though the frequency of BM monocytes remained constant after HFDs when compared to LFD (Figure 5F), HFD-SFAs, but not HFDs enriched in MUFAs, disturbed the relative proportion of the two principal monocyte subsets: Ly6C\(^+\) monocytes were increased (Figure 5G), and Ly6C\(^-\) monocytes were decreased (Figure 5H).

2.4. Effects of Fatty Acids in HFDs on Hematopoietic Stem and Progenitor Cells

The relationship between an HSC and its progeny as part of the hematopoietic hierarchy in the mouse system is depicted in Figure 6A. By using different antibody combination schemes, we first identified BM cells positive for the markers receptor tyrosine kinase, c-Kit (CD117) and stem cell antigen-1 (Sca-1), and negative for the selection markers of antigen-presenting cell lineages such as B220, CD3, CD4, CD8, CD11b, CD11c, Gr-1, and Ter-119. This pool of LKS (Lin\(^-\)cKit\(^+\)Sca1\(^+\)) cells that contained HSCs and progenitor (HPCs) cells[10] was refined into self-renewal with extended lifespan long-term hematopoietic stem cells (LT-HSCs) as Lin\(^-\)cKit\(^+\)Sca1\(^+\)CD34\(^-\)Flt3\(^-\) (FSM-like tyrosine kinase 3)\(^-\) cells (Figure 6B). This strategy included the identification of short-term HSCs (ST-HSCs) as Lin\(^-\)cKit\(^+\)Sca1\(^+\)CD34\(^+\)Flt3\(^+\) cells and multipotent progenitor (MPP) cells as Lin\(^-\)cKit\(^+\)Sca1\(^+\)CD34\(^+\)Flt3\(^-\) cells, which represent cell types of BM compartments immediately downstream of LT-HSCs. Within the LK (Lin\(^-\)cKit\(^+\)Sca1\(^-\)) pool, committed progenitor populations of oligopotent common myeloid progenitor (CMP) cells (Lin\(^-\)cKit\(^+\)Sca1\(^+\)CD34\(^+\)CD16/32\(^-\)) and of bivalent granulocyte-macrophage progenitor (GMP) cells (Lin\(^-\)cKit\(^+\)Sca1\(^+\)CD34\(^+\)CD16/32\(^+\)) and megakaryocyte-erythroid progenitor (MEP) cells (Lin\(^-\)cKit\(^+\)Sca1\(^+\)CD34\(^-\)CD16/32\(^-\)) and of common lymphoid progenitor (CLP) cells (Lin\(^-\)cKit\(^-\)Sca1\(^-\)Flt3\(^-\)CD127\(^-\)) were also pinpointed. The proportion of primitive LKS cells in the BM remained unaffected by HFDs compared to LFD (Figure 7A). However, HFDs enriched in MUFAs increased the proportion of LT-HSCs (Figure 7B) and decreased that of MPPs (Figure 7C). All HFDs led to a decline in the proportion of LK cells, and this effect was larger after HFD-SFAs (Figure 7D). There were no significant differences in the relative abundance of CMP cells when fed any diet (Figure 7E) or in the proportion of MEP cells (Figure 7F). In contrast, HFDs enriched in MUFAs increased the proportion of GMP cells (Figure 7G) and decreased that of CLP cells (Figure 7H). No significant differences were observed between HFD-MUFAs and HFD-MUFAs+EPA+DHA.

3. Discussion

Fatty acids are essential regulators of bone metabolism[35] and skeletal homeostasis.[36] When fatty acids are incorporated to a high extent into a diet (HFD), they impact the overall number of circulating[37] and marginal pools[19] of mature hematopoietic cells in mice. From these data, it is tempting to speculate that HFDs may also result in the regulation of HSC maturation in the BM niche. If it existed, we further hypothesized that this effect would be associated with the type of predominant fatty acids in the HFD.

Building from previous research reporting that MUFA in HFDs, in sharp contrast to SFAs, favor the homeostasis and a healthy expansion of eWAT in a mouse model of metabolic syndrome[38] we now show that HFDs enriched in MUFA or SFAs have distinct impacts within multilineage progeny of myeloid and lymphoid cells in a polygenic developmental model of HFD-induced obesity. All HFDs tested induced weight gain, increased adiposity, promoted dyslipidemia, and led to dysregulation of key hub genes involved in inflammation and energy balance in eWAT. However, such an obese phenotype became largely less disruptive after HFDs enriched in MUFA than after HFD-SFAs. For example, by indirect evidence of Ucp1 and Dio2 gene expression, it may be suggested that MUFAs could pave the way to restrict the fall in energy expenditure in white adipocytes. The expression levels of the Cebpb gene may also suggest that adipose tissue inflammation could not be required at all for the increase in adiposity after HFDs enriched in MUFA. Furthermore, while HFD-SFAs resulted in a decrease in circulating T cells in parallel with an increase in circulating Ly6C\(^+\) monocytes, HFDs enriched in MUFA increased circulating neutrophils and the HFD with added EPA and DHA also reduced circulating Ly6C\(^+\) monocytes below the values observed with LFD. Decreased circulating T cells have been documented with the progression of obesity induced by lard-based HFDs.[11] The SFA palmitic acid has been reported to stimulate a pro-inflammatory phenotype in T cells and an aberrant T cell infiltration of non-lymphoid tissues both in humans[39] and mice[40] and to be detrimental for cultured T cell survival.[41] Contrary to these effects, the MUFAs oleic acid has been shown to promote the formation of lipid droplets in a constant state of flux, resulting from an active cycle of lipolysis and re-esterification to provide fatty acids when needed for T cell development.[42] Recent studies on mice fed a chow diet supplemented with salmon oil for 3 weeks following allogeneic immunization have emphasized the role of EPA and DHA in decreasing the trafficking of pro-inflammatory T cells to fat depots.[43] Therefore, our study may support the hypothesis that dietary MUFA, EPA, and DHA together are helpful in creating a homeostatic environment for sustaining T cells in the circulation and for suppressing the pro-inflammatory programming of monocytes even under a chronic HFD challenge.

A number of studies have revealed that the circulating pool of neutrophils may be increased in conditions that do not promote explicit priming stimuli.[44] Contrarily to some predictions that a high number of neutrophils in the blood may be regarded as a sign of inflammation or infection, emerging evidence has revealed the ability of menhaden oil that is generously incorporated (38% of total calories) into a HFD to increase survival and decrease renal bacterial load in a mouse model of experimental sepsis through the selective enhancement of neutrophil frequency in blood and BM[45] and in spleen[19] prior to infection and to any evidence of inflammation. Similar findings were reported when menhaden oil was replaced by a commercial product containing...
Figure 6. Analysis of hematopoietic stem and progenitor cells in bone marrow of wild-type C57BL/6J mice. (A) Diagram showing the cell types differentiated within LKS (Lin−c-Kit+Sca1+) and LK (Lin−c-Kit+Sca1−) populations as well as their immunophenotypes based on the expression of CD34, Flt3, CD16/32, and CD127 markers. (B) An example of the gating strategy used to identify LT-HSC (Lin−c-Kit+Sca1+CD34−Flt3−), ST-HSC (Lin−c-Kit+Sca1+CD34+Flt3−), MPP (Lin−c-Kit+Sca1+CD34+Flt3+), CMP (Lin−c-Kit+Sca1−CD34+CD16/32−), GMP (Lin−c-Kit+Sca1−CD34+CD16/32+), MEP (Lin−c-Kit+Sca1−CD34−CD16/32−), and CLP (Lin−c-Kit+/LoSca1−Flt3+CD127+) cells in bone marrow. Debris (SSC-A vs FSC-A) and doublets (FSC-H vs FSC-A) were excluded. Frequency of cells in some sub-gates is expressed as percentage of live cells. CD, cluster of differentiation; FSC-A, forward scatter area; FSC-H, forward scatter height; SSC-A, side scatter area.
Figure 7. High-fat diets enriched in SFAs (anhydrous butter, HFD-SFAs), MUFAs (olive oil, HFD-MUFAs) or MUFAs (olive oil) fortified with EPA and DHA (HFD-MUFAs+EPA+DHA) on hematopoietic stem and progenitor cells in bone marrow of wild-type C57BL/6J mice. The percentages of (A) LKS, (B) LT-HSC, (C) MPP, (D) LK, (E) CMP, (F) MEP, (G) GMP, and (H) CLP cells at the end of 20-week dietary regimens. Viable cells (7-AAD−Annexin V−) were only considered. Values are individual data points ± SD; n = 20/group. Differences between groups were assessed by two-way ANOVA with Tukey’s post hoc test. ***P < 0.001; **P < 0.01; *P < 0.05 (compared to all other study groups and as indicated). CLP, common lymphoid progenitor; CMP, common myeloid progenitor; GMP, granulocyte-macrophage progenitor; LK, Lin−cKit+ Sca1− cell; LKS, Lin−cKit+ Sca1+ cell; LT-HSC, long-term hematopoietic stem cell; MPP, multipotent progenitor; MEP, megakaryocyte-erythroid progenitor.
high amounts of EPA and DHA ethyl esters in a HFD. Different from these overfeeding studies, we have observed that the addition of a more realistic dose of EPA and DHA to a HFD formulated with olive oil as the main source of fat had little if any impact on neutrophil frequency in blood, probably unveiling the yet unknown role of dietary MUFAs in supporting or optimizing immune function and surveillance in this compartment during obesity, even in the presence of EPA and DHA. The mediators that can alert the host defense system in a fatty acid-dependent manner and that are not committed to major inflammatory responses remain to be elucidated. These findings evoke the biological process of trained immunity, which is under study in our laboratory.

The observation of increased circulating neutrophil number after HFDs enriched in MUFAs was also found in the spleen. Moreover, HFDs enriched in MUFAs increased myeloid-derived splenic Ly6C\(^{hi}\) monocytes, thus explaining the concomitant reduction in this subset in the blood, particularly after HFD-MUFAs fortified with EPA and DHA. In previous studies, the spleen and most notably its peri-marginal zone areas have been shown to be colonized by neutrophils for homeostatic reasons in healthy mammals, including humans, monkeys, and mice. More recently, the increased accumulation of neutrophils in the peri-marginal zone areas of the spleen has been reported to occur in mice fed a HFD enriched with high levels of menhaden oil after 4 weeks. The spleen is also a compartmental reservoir of extramedullary monocytes. Interestingly, although Ly6C\(^{hi}\) monocytes often synthesize and secrete inflammatory mediators, they can accumulate in the spleen in the absence of demands for rapid-onset inflammation, and when this occurs, they exit the spleen accompanied by Ly6C\(^{lo}\) monocytes with healing properties to inflamed tissue; this pattern is independent of that of splenic neutrophils. If the existence of neutrophils and monocytes, with HFD-SFAs decreasing neutrophils and disturbing the ratio of Ly6C\(^{hi}\) to Ly6C\(^{lo}\) monocytes in favor of the Ly6C\(^{lo}\) subset, while HFDs enriched in MUFAs only increased neutrophils relative to the values observed with the LFD. In previous studies with mice fed a 45% lard-based HFD for different periods up to 6 weeks, a progressive increase in the number of lymphocytes in BM was noticed, without signs of inflammation in this compartment. These discrepancies can be explained, at least in part, by the greater fat content in our HFDs (53% of calories) and by the longer feeding time (20 weeks) in our protocol. More in line with our own observations, recent studies in mice fed a 60% lard-based HFD for different feeding periods, even longer than 20 weeks, have reported disturbance in the hematopoietic system giving rise to defective lymphocyte populations and to myeloid progenitors that sustain the production of Ly6C\(^{hi}\) monocytes poised for innate immune activation as well as a null pro-inflammatory phenotype in the BM and altered stemness and differentiation in the BM stroma. Hence, it is tentatively speculated that HFD-induced myelopoiesis may be dissected by the composition of fatty acids in HFDs, with SFAs involved in the guidance of monopoiesis and MUFAs in the orchestration of granulopoiesis. Intriguingly, both cell fate decisions are known to be mutually exclusive. Recruit- ment of classical monocytes during chronic inflammation in adipose depots, which is commonly seen in the obesity induced by HFDs enriched in SFAs, has been proposed to be a determinant for BM monocyte output on demand. Hypercholes- terolemia, which is provoked in most cases by HFDs enriched in SFAs, has also been shown to be associated with peripheral Ly6C\(^{hi}\) monocytes. With the aim of gaining insight into the role of SFAs and MUFAs on lineage commitment in BM during obesity, we found that HFD-SFAs decreased the percentage of LK cells when compared to the effects of a LFD and HFDs enriched in MUFAs, which may suggest that SFAs can significantly block the survival or expansion of these cells during obesity. A recent description of a feed-forward mechanism in lard-based
HFD-induced obesity such that inflamed adipose tissue macrophages continuously instigate the production of monocytes could be behind the possible exhaustion of the LK cell pool.\(^{[18]}\) The selective reduction of LK cells has been observed in the BM of mice lacking the ability of long term multilineage engraftment,\(^{[59]}\) consistent with LK cells also being involved in the immune reconstitution of damaged BM.\(^{[60]}\) In addition, MUFAs in HFDs targeted at the root of the branching family tree, where the most immature pool of HSCs is endowed with the highest self-renewal potential (LT-HSCs and MPP cells) and the most restricted progenitors are in charge of myelopoiesis (GMP cells) and lymphopoiesis (CLP cells), increase the percentage of LT-HSCs and bivalent GMP cells and decrease that of MPP and CLP cells. These findings emphasize the regulatory role of MUFAs in the balance between LT-HSC self-renewal and differentiation during obesity, probably promoting a shift towards self-renewing divisions of LT-HSCs and an adhesive environment. Whether this overrepresentation of LT-HSCs led to a depletion of MPP and CLP cells as a feedback compensatory mechanism has yet to be seen. The observed phenotypic signature of LT-HSC and MPP cell populations representative of mice fed HFDs enriched in MUFAs could be associated with the fitness of the HSC compartment.\(^{[17]}\) This is an interesting issue, as a decrease in the number of LT-HSCs in the BM is common in the obesity induced by lard-based HFDs\(^{[18]}\) and in aged mice,\(^{[61]}\) which heightens the vulnerability of LT-HSCs to DNA-damaging processes.\(^{[62]}\) Therefore, the effect of enhanced retention and preservation of LT-HSCs in the BM may be crucial to sustain life-long hematopoiesis in obesity if the HFD is mainly composed of MUFAs instead of SFAs.

Additionally, oleic acid but not another MUFA (palmitoleic acid) or PUFA has been recently shown to be a requirement for the induction of innate immunity and resistance to infection in the nematode *Caenorhabditis elegans*,\(^{[63]}\) which could reveal robust conservation of the role of oleic acid in the heavy interplay with the immune system among model organisms in the animal kingdom.

Despite EPA and DHA were used to fortify the HFD based on olive oil, many of the selected outcomes of this study did not differ between mice that received the HFD-MUFAs and those that received the HFD-MUFAs + EPA + DHA. We do not rule out a hierarchical usefulness of oleic acid from olive oil over EPA and DHA in a physiological context in which HFDs exhibited canonical proportions of SFAs, MUFAs, and PUFAs. The dose of EPA and DHA was consistent with previous studies,\(^{[64]}\) however our understanding of the events underpinning the impact of these long-chain omega-3 fatty acids on hematopoietic cells during obesity is far from complete and novel experimental strategies will require further refinement.

In the face of these promising results, some limitations of our study should be mentioned. First, our exploratory analysis does not inform whether dietary fatty acids ingested at lower doses than those employed in HFDs, that is, in the context of a non-obesogenic diet, have the potential to influence the hematopoietic system. Second, because hematopoiesis is a multi-regulated process, the levels at which dietary fatty acids exert their effects remain unclear; for example, whether the regulation occurs via direct interaction of dietary fatty acids with hematopoietic cells or with selective BM niches or indirectly in an autocrine or a paracrine manner by way of fatty-acid-mediated generation of

Figure 8. A summary of hematopoietic cells mainly altered in bone marrow, blood, and spleen during the obesity induced by high-fat diets enriched in SFAs (anhydrous butter, HFD-SFAs), MUFAs (olive oil, HFD-MUFAs), or MUFAs (olive oil) fortified with EPA and DHA (HFD-MUFAs+EPA+DHA) in wild-type C57BL/6J mice.
specific hormones or cytokines within the medullary tissue or in extramedullary sites remains unclear. Third, quantification of absolute cell numbers per sample volume was not achieved in spleen and BM, and thus, it remains unclear whether the overall cellularity of these tissues was affected by fatty acids in HFDs. Further studies are needed to clarify these points.

In conclusion, these studies delineate a functional role for fatty acids in HFDs to guide HSC maturation in the BM niche and the abundance of mature hematopoietic populations in the blood and spleen. Interestingly, these effects were traced to the type of predominant fatty acids in HFDs. These data support a complex model in which the reshaping of fatty acid pools with MUFAs from the diet serves to manipulate the generation and trafficking of immune cells biased during obesity (Figure 8). This perception of fatty acids in sites for hematopoiesis could have significant implications for developing novel strategies targeting the diverse metabolism of dietary fatty acids in the BM and extramedullary compartments to combat the HFD-induced dysfunctional immune system.

4. Experimental Section

Mice and Diets: Six-week-old wild-type C57BL/6 male mice (Charles River Laboratorte, Ecully, France) were used for this study. Mice were maintained in a light/dark- (12 h light/12 h dark), temperature- (22 ± 1 °C), and humidity- (50–60%) controlled room under specific pathogen-free conditions, fed a LFD (10% of energy as fat from soybean oil) (Table S1, Supporting Information) ad libitum and had free access to drinking water for 2 weeks to adapt to the Mouse Facility of the Centro Andaluz de Biología del Desarrollo (CABD, Seville). Then, animals were divided into four experimental groups (n = 20/group) and fed the LFD (control) or HFDs based on the LFD with an additional 43% of energy as fat from anhydrous butter (HFD-SFAs), olive oil (HFD-MUFAs), or olive oil (40% of energy) plus EPA + DHA (3% of energy) in the form of ethyl esters (HFD-MUFAs+EPA+DHA) (Table S1, Supporting Information) for 20 weeks. Diets and drinking water were refreshed every week. Olive oil was devoid of minor constituents as obtained by physical refining of virgin olive oil in a discontinuous deodorizer that used nitrogen as the 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 (Table S2, Supporting Information) was determined via lipid extraction, saponification, and capillary GC.[65] Carbohydrate was used to adjust the total energy content. All diets had low cholesterol (0.01%), were prepared by SAFE (Augy, France) and were presented as pellets to the animals. Body mass and food and water intake were recorded weekly. At the end of the experiments, animals were euthanized at the beginning of the light cycle, blood was collected by cardiac puncture using heparinized syringes and tubes (MiniCoulter™, France) and were presented as pellets to the animals. Body mass and food intake were recorded weekly. At the end of the experiments, all animals were euthanized by using the standard 2% isoflurane method. All data were normalized to the content of housekeeping gene and expressed as a percentage of the control.

Statistics: All results are expressed as the means with error bars reflecting SD. n represents the number of animals per experiment.

DNA Isolation and qRT-PCR Analysis: eWAT tissue samples were crushed with liquid nitrogen in a precooled mortar and pestle to recover a fine powder. The fine powder was collected in Eppendorf tubes and further processed. Total RNA was then extracted using Trisure Reagent (Bioline, London, UK) and purified through spin columns of the Direct-zol MiniPrep Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer’s recommendation. RNA was quantified in a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). RNA quality was assessed using the QIAxcel Advanced System (QIAGEN) and QI-Axcel ScreenCel software. The RIS number for all RNA preparations was 7. RNA (1 µg) was subjected to reverse transcription (iScript, Bio-Rad, Madrid, Spain). An amount of 20 ng of the resulting cDNA was used as a template for real-time PCR amplification. The mRNA levels of specific genes were determined in a CFX96 system (Bio-Rad). For each PCR, cDNA template was added to Brilliant SYBR green QPCR Supermix (Bio-Rad) containing the primer pairs for the corresponding gene. The glyceraldehyde-3-phosphate dehydrogenase (Gapdh) housekeeping gene was used for normalization. Sequences for qRT-PCR primers can be found in Table S4, Supporting Information. All amplification reactions were performed in triplicate, and average threshold cycle (Ct) numbers of the triplicates were used to calculate the relative mRNA expression of candidate genes. The magnitude of change in mRNA expression for candidate genes was calculated by using the standard 2−(ΔΔCt) method. All data were normalized to the content of housekeeping gene and expressed as a percentage of the control.

Statistics: All results are expressed as the means with error bars reflecting SD. n represents the number of animals per experiment.
Differences between groups were assessed by two-way ANOVA with Tukey’s post hoc test.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements
This work was supported by a grant from The Spanish Ministry of Science, Innovation and Universities (AGL2016-80852-R). A.L.-C. had a contract associated with AGL2016-80852-R during the experimental part of this work. S.L. acknowledges financial support by a contract from the “Own Research Plan” of the University of Seville (VPPI-US) cofounded by the European Social Fund. M.A.R. acknowledges financial support from CSIC/Juan de la Cierva (FJCI-2017-33132). The authors thank Core Facilities for Oil Extraction and Refining and Cell Biology Unit at the Instituto de la Grasa (CSIC, Seville) and Mouse Facility at the CABD (UPO/Junta de Andalucia/CSIC, Seville) for their assistance during the fulfillment of this study.

Conflict of Interest
The authors declare no conflict of interest.

Author Contributions
A.L.-C. and M.M. authors contributed equally to this work. F.J.G.M. and R.A. designed research; A.L., M.M., S.L., M.C.M., M.A.R., J.A.P.S., and R.A. performed experiments, and F.J.G.M. and R.A. wrote the main manuscript. All authors analyzed and discussed the data, and read and approved the final manuscript.

Data Availability Statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords
dietary fatty acids, hemopoiesis, MUFAs, obesity, progenitor cells, stem cells

Received: December 21, 2020
Revised: May 27, 2021
Published online:

[1] World Health Organization, World Health Organ. Tech. Rep. Ser. 2000, 894, 1.
[2] M. J. Muller, C. Geisler, Eur. J. Clin. Nutr. 2017, 71, 1256.
[3] R. Estruch, E. Ros, J. Salas-Salvado, M. I. Covas, D. Corella, F. Aros, E. Gomez-Gracia, V. Ruiz-Gutierrez, M. Fiol, J. Lapetra, R. M. Lamuela-Raventos, L. Serra-Majem, X. Pinto, J. Basora, M. A. Muñoz, J. V. Sorlí, J. A. Martínez, A. M. Martínez-Gonzalez, N. Engl. J. Med. 2013, 368, 1279.
[4] C. Capurso, M. Massaro, E. Scoditti, G. Vendemiale, A. Capurso, Vasc. Pharmacol. 2014, 63, 118.
[5] H. Tutunchi, A. Ostadrarhimi, M. Saghafi-Asl, Adv. Nutr. 2020, 11, 864.
[6] A. C. Skulas-Ray, P. W. F. Wilson, W. S. Harris, E. A. Brinton, P. M. Kris-Etherton, Ch. K. Richter, T. A. Jacobson, M. B. Engler, M. Miller, J. G. Robinson, C. B. Blum, D. Rodriguez-Leyva, S. D. de Ferranti, F. K. Welty, Council Clin. Cardiol. Circ. 2019, 140, e673.
[7] R. D. C. da Cunha de Sa, M. M. Cruz, T. M. de Farias, V. S. da Silva, J. de Jesus Simao, M. M. Telles, M. I. C. Alonso-Vale, Physiol. Rep. 2020, 8, e14380.
[8] A. Benova, M. Tencerova, Front. Endocrinol. 2020, 11, 294.
[9] M. Kashimura, Int. J. Hematol. 2020, 112, 147.
[10] R. Kumar, P. S. Godavarthy, D. S. Krause, J. Cell Sci. 2018, 131, jcs201707.
[11] H. Yang, Y. Youm, B. Vandanmagsar, A. Ravussin, J. M. Gimble, F. Greenway, J. M. Stephens, R. L. Mynatt, V. D. Dixit, J. Immunol. 2010, 185, 1836.
[12] M. D. Trottier, A. Naaz, Y. Li, P. J. Fraker, Proc. Natl. Acad. Sci. USA 2012, 109, 7622.
[13] K. Singer, J. DelProposto, D. L. Morris, B. T. Mergian, N. Maley, K. Won Cho, L. Geletka, P. Subbaiah, L. Muir, G. Martinez-Santibanez, C. N. Lumeng, Mol. Metab. 2014, 3, 664.
[14] B. J. Adler, K. Kaushansky, C. T. Rubin, Nat. Rev. Endocrinol. 2014, 10, 737.
[15] L. Wu, V. V. Parekh, J. Hsiao, D. Kitamura, L. Van Kaaer, Proc. Natl. Acad. Sci. USA 2014, 111, E4638.
[16] M. Tencerova, F. Figeac, N. Ditzel, H. Taipaleenmaki, T. K. Nielsen, M. Kassem, J. Bone Miner. Res. 2018, 33, 1154.
[17] J. Lee, V. Govindarajah, B. Goddard, A. Hinge, D. E. Muench, M. Filippi, B. Aronow, J. A. Cancelas, N. Salomonis, H. L. Grimes, D. Rey, Exp. Med. 2018, 215, 627.
[18] A. Liu, M. Chen, R. Kumar, M. Stefanovic-Racic, R. M. O’Doherty, Y. Ding, W. Jahnne-Dechent, L. Borghesi, Nat. Commun. 2018, 9, 708.
[19] S. L. Svatn, S. Gutierrez, M. A. Ulleryd, I. Nookaew, V. Osfa, F. Beckham, S. Nilsson, A. Karlsson, J. Jansson, M. E. Johansson, Infect. Immun. 2019, 87, e00270.
[20] Z. Ren, X. Gu, J. Fang, D. Cai, Z. Zuo, S. Liang, H. Cui, J. Deng, X. Ma, Y. Geng, M. Zhang, Y. Xie, G. Ye, L. Gou, Y. Hu, Sci. Rep. 2020, 10, 5109.
[21] Y. Liu, X. Lu, X. Li, P. Du, G. Qin, Mol. Immunol. 2020, 117, 139.
[22] K. J. Mamounis, A. Yasrebi, T. A. Roepke, J. Nutr. Biochem. 2017, 40, 122.
[23] R. Kubant, A. N. Poon, D. Sanchez-Hernandez, A. F. Domenichelli, P. S. P. Huot, E. Pannia, C. E. Cho, S. Hunschede, R. P. Bazinet, G. H. Anderson, Nutr. Diabetes. 2015, 5, e188.
[24] S. L. Svatn, L. Varemo, B. G. Gabrielson, E. Peris, I. Nookaew, L. Grahmno, A. Sandberg, J. Wernerstedt Asterholm, J. Jansson, M. E. Johansson, PLoS One 2016, 11, e0155099.
[25] H. Oguro, Front. Endocrinol. 2019, 10, 204.
[26] I. J. Neelan, P. Poirier, J. Despres, Circulation 2018, 137, 1391.
[27] C. Liu, K. E. Broe, Y. Zhou, S. K. Boyd, L. Adrienne Cupples, M. T. Hannan, E. Lim, R. R. McLean, E. J. Samelson, M. L. Bouxsein, D. P. Kiel, J. Bone Miner. Res. 2017, 32, 143.
[28] M. S. Han, A. White, R. J. Perry, J. Camporez, J. Hidalgo, G. I. Shulman, R. J. Davis, Proc. Natl. Acad. Sci. USA. 2020, 117, 2751.
[29] S. Motoyama, H. Yamada, K. Yamamoto, N. Nakama, K. Terada, M. Kikai, N. Wada, M. Saburi, T. Sugimoto, H. Kubota, D. Miyawaki, D. Kami, T. Ogata, M. Ibi, C. Yabe-Nishimura, S. Matoba, Cells. 2020, 9, 996.
[30] S. M. Rahman, R. C. Janssen, M. Choudhury, K. Baquero, R. M. Aikens, B. A. de la Houssaye, J. E. Friedman, J. Biol. Chem. 2012, 287, 34349.
[31] R. Siersbaek, R. Nielsen, S. Mandrup, FEBS Lett. 2010, 584, 3242.
[32] N. Petrovic, T. B. Walden, I. C. Shabalina, J. A. Timmons, B. Cannon, J. Nedergaard, J. Biol. Chem. 2010, 285, 7153.
