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# Short-Term Western Diet Causes Rapid and Lasting Alterations of Bone Marrow Physiology

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Dietary parameters have a strong influence on overall health in various ways. For instance, it affects the physiology of blood vessels. Consumption of western diet (WD) dysregulates the arterial homeostasis and promotes the development of atherosclerotic plaques in the intimal layer of arteries.[1] Interestingly, it was recently described that WD also alters the composition and function of blood vessels in the bone marrow,[2] structures which are considered to play a pivotal role in the microenvironment or niche that tightly controls hematopoiesis.[3] Endothelial cells as well as perivascular cells such as pericytes, mesenchymal stem cells, and CXCL12-abundant reticular cells that are found in bone marrow arteriolar and sinusoidal vessels regulate the maintenance of hematopoietic stem cells (HSCs).[4,5] Thus, modifications of the architecture and/or the features of the bone marrow vascular network may have dramatic effects on hematopoiesis. Long-term and permanent WD was shown to modify the anatomy of the vascular bone marrow niche and HSC biology.[2] It remains unknown whether a short-term and acute WD regimen would be sufficient to initiate modifications of the bone marrow physiology and whether these alterations would be reversible.

We therefore investigated the effects of different short-term WD conditions (►**Fig. 1A**) on the bone marrow vasculature and hematopoiesis in *Apoe*$^{-/-}$ hypercholesterolemic mice (see also ►**Supplementary Methods** [available in the online version]). First, microscopic three-dimensional visualization of optically cleared bones allowed us to assess the density of the bone marrow vessel network using laminin as a marker for vessel density and early remodeling (►**Fig. 1B** and ►**Supplementary Fig. S1A**, available in the online version), revealing that 4 weeks' regimen of WD (chronic WD group) promoted the remodeling of arterioles (laminin$^{high}$ endoglin$^{-}$)[6] but not sinusoids (laminin$^{+}$ endoglin$^{+}$; ►**Fig. 1B, C** and ►**Supplementary Fig. S1B, C**, available in the online version). Moreover, 1 week of WD only (late WD group) was already sufficient to induce a similar effect with upregulated laminin expression (►**Fig. 1B, C** and ►**Supplementary Fig. S1B, C**, available in the online version), suggesting that remodeling of the bone marrow arterioles by WD is an acute and rapid process. Next, we tested whether these fast changes observed in the arterioles were reversible. To this end, WD was fed to a group of mice for 1 week only and thereafter was replaced by a chow diet for the following 3 weeks (early WD group). Surprisingly, the enhanced laminin expression in bone marrow arterioles was maintained after the interruption of WD for the remaining weeks. Of note, none of the different conditions (chronic, late, and early WD) altered the density of the bone marrow sinusoids (►**Fig. 1B** and ►**Supplementary Fig. S1B, C**, available in the online version). Altogether, these findings highlight that short-term WD not only induces a fast remodeling of the bone marrow arterioles, but also that these alterations remain present for a prolonged period of time.

The bone marrow vascular network was suggested to serve as a niche and under steady state, HSCs reside in close

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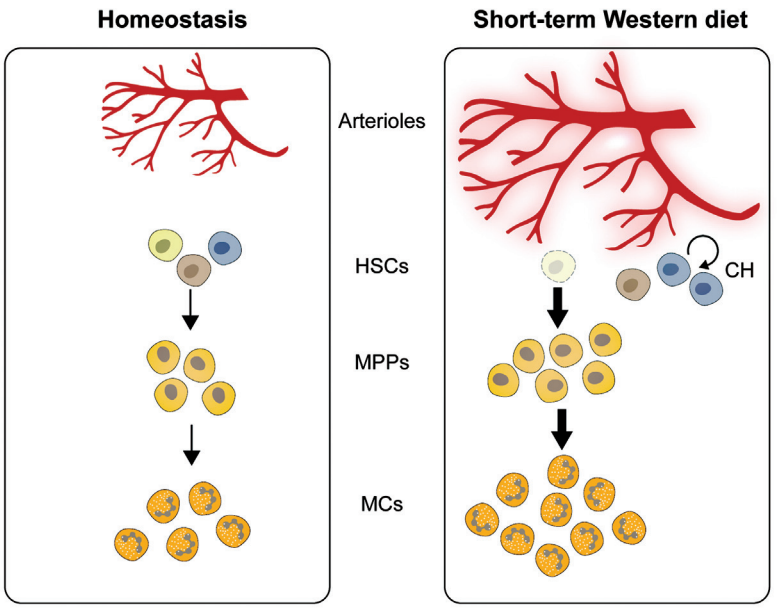


**Visual summary.** Short-term western diet (WD) induces rapid remodeling of bone marrow arterioles, and intriguingly, these alterations persist even after the cessation of WD. Notably, distance between hematopoietic stem cells (HSCs) and arterioles following short-term WD is reduced. This proximity is accompanied by a reduction in HSC counts, an elevated number of progenitor cells (MMPs), and an increased production of myeloid cells (MCs). Furthermore, the remaining HSCs proliferate, potentially rendering them more susceptible to clonal hematopoiesis (CH). Our findings demonstrate that short-term WD exerts profound effects on bone marrow physiology, which have long-lasting consequences.

vicinity of arterioles or sinusoids.[4] Since arteriolar laminin expression was already increased by short-term WD, we investigated the relationship of HSCs with these vessels.[7] Three-dimensional computation of HSC–vessel spatial relationships at a single-cell level revealed that permanent WD strongly increased the proximity between HSCs ($Lin^-$, $CD150^+$, $CD48^-$)[7,8] and arterioles (►**Fig. 1D**). Although less pronounced, we could detect a similar effect after 1 week of WD at late or early time point (►**Fig. 1D**). Given the importance of bone marrow niche on HSC homeostasis, we subsequently evaluated the contribution of the short-term WD on HSC counts. Flow cytometry analysis revealed that, in chronic, late, and early conditions of WD, numbers of HSCs were decreased (►**Fig. 1E**). We hypothesized that this reduction was the consequence of a deterioration in the maintenance of HSCs. However, we did not observe any changes in apoptosis (►**Supplementary Fig. S1D**, available in the online version). We then reasoned that short-term WD induced the differentiation of HSCs into a downstream population, namely multipotent progenitors (MPPs).[9] Indeed, the numbers of myeloid-biased MPP (a.k.a. MPP3)[9] were greater in all groups of mice that were fed a short-term WD compared to control mice (►**Fig. 1F**), whereas proportions of lymphoid-primed MPP (a.k.a. MPP4)[9] remained unchanged (►**Supplementary Fig. S1E**, available in the online version). We then investigated whether the expansion of the myeloid-biased MPP population correlated with an increase in myeloid cells. The different WD conditions led to elevated numbers of neutrophils and monocytes in the bone marrow (►**Fig. 1G, H**). Subsequently, we examined whether this expansion had an impact on blood cell counts. Circulating monocytes showed an increase in all WD conditions (►**Fig. 1J**), while higher neutrophil counts were only detected in the late WD group (►**Fig. 1I**). To understand the mechanisms involved in neutrophil and monocyte mobilization, we assessed the plasma levels of the chemokines CXCL1[10] and CCL2.[11] We observed higher levels of CXCL1 in the plasma of the late WD group (►**Supplementary Fig. S1F**, available in the online version), providing an explanation for the increased presence of neutrophils in circulation within this group. However, CCL2 levels remained unaffected in all WD groups (►**Supplementary Fig. S1G**, available in the online version), suggesting the involvement of another mechanism in their mobilization. Together, these findings indicate that short-term WD after 1 week (late WD group) induces an accelerated differentiation of HSC to MPP3 and led to an increase of myeloid cell production. Interestingly, our data further imply that these effects are long lasting since the upregulation of myelopoiesis could still be observed even after suspension of WD (early WD group).

HSCs are by definition key in maintaining proper hematopoiesis, and a loss of HSCs can have significant consequences on the production of hematopoietic cells. In our study, we observed a significant decrease in HSC numbers across all short-term WD groups (►**Fig. 1E**). To further investigate their maintenance, we examined whether the proliferation of the remaining HSCs was affected. Utilizing Ki67 staining, we found that 62% of HSCs in $Apoe^{-/-}$ mice on a chronic WD were positive, whereas only 24% of HSCs in chow-fed $Apoe^{-/-}$ mice showed positivity (►**Fig. 1K**). Notably, we also observed an

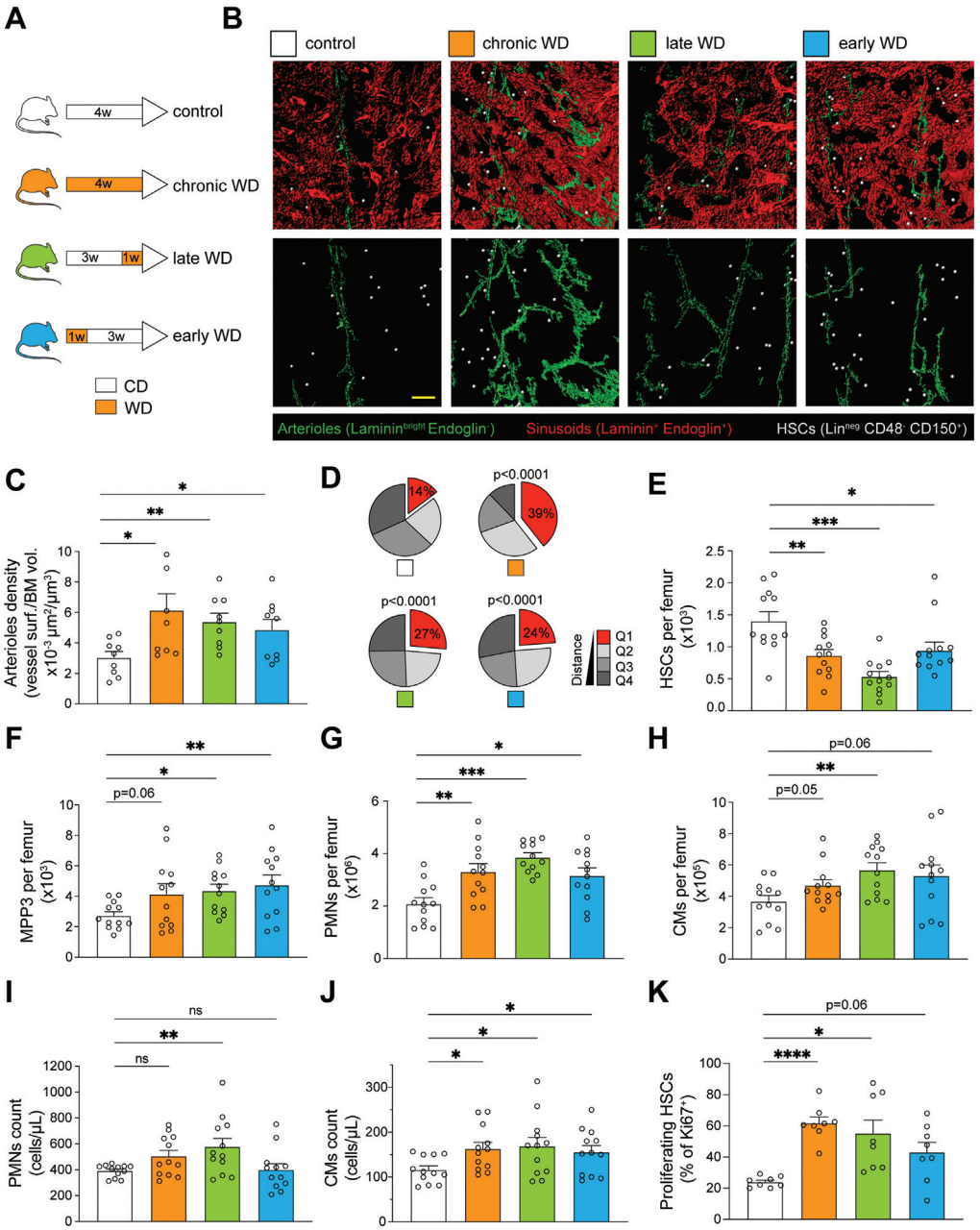


**Fig. 1** Short-term WD alters bone marrow physiology. (**A**) Schematic representation of dietary interventions. *Apoe*$^{-/-}$ mice were fed either a chow diet (CD) for 4 weeks (control), western diet (WD) for 4 weeks (chronic WD), 3 weeks of CD followed by 1 week of WD (late WD) or 1 week of WD followed by 3 weeks of CD (early WD). (**B**) Representative confocal microscopy 3D reconstruction of HSC (Lin$^-$ CD48$^-$ CD150$^+$, white spheres), sinusoids (laminin$^+$ endoglin$^+$, red structure), and arterioles (laminin$^{high}$ endoglin$^-$, green structure) in optically cleared whole mount bone marrow from *Apoe*$^{-/-}$ fed CD (control) or WD (chronic, late and early groups). Scale bar = 50 µm. (**C**) Quantification of the density of bone marrow arterioles. $n = 9$ random field of views pooled from three mice per group. (**D**) Distance between HSCs and arterioles in bone marrow. Distribution quartiles were defined as Q1 ≤11.67 µm, Q2 ≤35.2 µm, Q3 ≤75.66 µm, and Q4 ≥75.67 µm. $n = 601$ HSCs (control group), $n = 442$ HSCs (chronic group), $n = 405$ HSCs (late group), and $n = 455$ HSCs (early group) from three mice per group. $p < 0.001$ ($\chi^2$ test). (**E**) Numbers of HSCs (Lin$^-$ Sca1$^+$ c-Kit$^+$ CD150$^+$ CD48$^-$) in the bone marrow of *Apoe*$^{-/-}$ mice fed with CD (control) or WD (chronic, late, and early groups) assessed by flow cytometry. (**F**) Numbers of myeloid-biased MPP3s (Lin$^-$ Sca1$^+$ c-Kit$^+$ CD150$^-$ CD48$^+$ Flt3$^-$) in the bone marrow of *Apoe*$^{-/-}$ mice fed with CD (control) or WD (chronic, late, and early groups). (**G**) Numbers of polymorphonuclear neutrophils (CD45$^+$ CD11b$^+$ CD115$^-$ Ly6G$^+$) in the bone marrow of *Apoe*$^{-/-}$ mice fed with CD (control) or WD (chronic, late, and early groups). (**H**) Numbers of classical monocytes (CD45$^+$ CD11b$^+$ CD115$^+$ Ly6C$^+$) in the bone marrow of *Apoe*$^{-/-}$ mice fed with CD (control) or WD (chronic, late, and early groups). (**I**) Numbers of polymorphonuclear neutrophils (CD45$^+$ CD11b$^+$ CD115$^-$ Ly6G$^+$) in the blood of *Apoe*$^{-/-}$ mice fed with CD (control) or WD (chronic, late, and early groups). (**J**) Numbers of classical monocytes (CD45$^+$ CD11b$^+$ CD115$^+$ Ly6C$^+$) in the blood of *Apoe*$^{-/-}$ mice fed with CD (control) or WD (chronic, late, and early groups). (**K**) Percentage of Ki67$^+$ HSCs Lin$^-$ Sca1$^+$ c-Kit$^+$ CD150$^+$ CD48$^-$) in the bone marrow of *Apoe*$^{-/-}$ mice fed with CD (control) or WD (chronic, late, and early groups). All experiments presented in panels (**C**) and (**E–J**) were performed with $n = 12$ mice per group from three independent experiments, except the experiment depicted in (**K**) that was performed with $n = 8$ mice per group from two independent experiments. Mean ± SEM. One-way ANOVA test. $^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. 3D, three-dimensional; HSCs, hematopoietic stem cells; SEM, standard error of the mean.

increased percentage of Ki67-positive HSCs in the late WD group (55% of HSCs) and the early WD group (43% of HSCs). Collectively, these findings suggest that, after differentiating into MPPs, the remaining HSCs undergo proliferation, likely to maintain their pool. Furthermore, this proliferation of HSCs occurs rapidly and continues over an extended period, as evidenced by increased HSC proliferation in both the late and early WD groups.

Clonal hematopoiesis (CH) is characterized by the proliferation of a specific clone of HSCs, with or without a genetic mutation, and is commonly associated with the aging process.[12] Certain somatic mutations in CH have been found to contribute to atherosclerosis by affecting the behavior of myeloid cells.[13] More recently, it has been shown that increased proliferation of HSCs by long-term WD in atherosclerosis condition accelerates the emergence of CH.[14] Our data suggest that short-term WD can trigger the initiation of CH, primarily through neutral drift and more specifically via a bottleneck effect, and potentially provides an explanation for the subsequent appearance of CH described with prolonged exposure to WD. Indeed, the initial drastic reduction of HSC pool size following short-term WD, especially observed after 1 week of WD (in the late group in ►**Fig. 1E**), leads to the constitution of a smaller number of HSC clones (38% of the initial pool). The expansion of these limited clones, that is already noticeable 3 weeks after the cessation of WD (early group in ►**Fig. 1E**), will inevitably promote the emergence of CH, regardless of genetic mutations. It is worth noting that even in a healthy hematopoietic system, small clones carrying mutations can exist.[15] If one of these clones harboring a mutation proliferate, it will then become over-represented in the context of WD-induced hematopoiesis.[14] Alternatively, as uncontrolled proliferation increases the likelihood of mutations, it raises the probability that one clone acquires driver mutations and dominates over others if there is a selective advantage for the variant.

The environment of the bone marrow niche plays an important role in hematopoiesis.[3,4] The function of the arterial niche for the maintenance of HSCs has been controversial. A proportion of HSCs was shown to associate with arterioles and molecular factors produced by vascular endothelial cells and perivascular cells as well as a microenvironment low in reactive oxygen species were proposed to maintain HSC quiescence.[16–19] In contradiction to these findings, it was also shown that quiescent HSCs were closely associated with sinusoids, while arterioles did not play a role in HSC maintenance.[7,20,21] Our findings suggest that a disturbed microenvironment centered around arterioles, and induced by a brief consumption of WD, results in the activation (differentiation and/or proliferation) of HSCs. In our study, laminin was utilized for the identification of bone marrow arterioles which showed a clear increase of this extracellular matrix protein upon WD stimulus. Interestingly, an altered production of laminin may be considered as an early hallmark of vascular remodeling.[22] Moreover, it was shown that bone marrow laminins serve as adhesive substrates[23] to HSCs and regulate their maintenance.[24] Potentially, additional changes in the activated bone marrow niches that occur in parallel of laminin expansion could influence the maintenance of HSCs. Nevertheless, it is conceivable that short-term WD activates bone marrow arterial endothelial and perivascular cells, which leads to an increase of laminin production in arterioles and consequently affects HSC biology. The exact reason behind the prolonged impact of WD on laminin levels after WD cessation remains uncertain. However, it is possible that the turnover of laminin, which relies on a delicate balance between synthesis and degradation processes,[25] might be influenced in this particular context. It is conceivable that cells surrounding arterioles could remain active for an extended period even after WD discontinuation and continue to produce laminin. Alternatively, the onset of laminin degradation, facilitated by enzymes like metalloproteinases and proteases, could be slower in this scenario.

In conclusion, our data show that a brief exposure to WD induces a rapid and sustained alteration of the bone marrow physiology. This may have deleterious effects in mounting an efficient immune response[26] and could have harmful consequences in the development of an inflammatory response.[13,14] Further research is required to understand the regulatory pathways involved in the sustained remodeling of bone marrow niches and its consequences on hematopoiesis.

### Authors' Contribution

M.B. designed and performed experiments, analyzed, and interpreted data; Z.M.-R. performed experiments, analyzed, and interpreted data; C.W. provided funding and intellectual input; R.T.A.M and J.D. provided funding, designed the research, interpreted data, and wrote the manuscript.

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### Conflict of Interest

None declared.

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