Abstract: Diet, exercise, stress, and sleep are receiving attention as environmental modifiers of chronic inflammatory diseases, including atherosclerosis, the culprit condition of myocardial infarction and stroke. Accumulating data indicate that psychosocial stress and a high-fat, high-cholesterol diet aggravate cardiovascular disease, whereas regular physical activity and healthy sleeping habits help prevent it. Here, we raise the possibility that inflammation-associated leukocyte production plays a causal role in lifestyle effects on atherosclerosis progression. Specifically, we explore whether and how potent real-life disease modifiers influence hematopoiesis’ molecular and cellular machinery. Lifestyle, we hypothesize, may rearrange hematopoietic topography, diverting production from the bone marrow to the periphery, thus propagating a quantitative and qualitative drift of the macrophage supply chain. These changes may involve progenitor-extrinsic and intrinsic communication nodes that connect organ systems along neuroimmune and immunometabolic axes, ultimately leading to an altered number and phenotype of lesional macrophages. We propose that, in conjunction with improved public health policy, future therapeutics could aim to modulate the quantitative and qualitative output, as well as the location, of the hematopoietic tree to decrease the risk of atherosclerosis complications. (Circ Res. 2015;116:884-894.
DOI: 10.1161/CIRCRESAHA.116.303550.)

Key Words: atherosclerosis ■ bone marrow ■ cell proliferation ■ diet ■ hematopoiesis ■ macrophages ■ monocytes ■ psychosocial, stress ■ sleep ■ spleen

In recent decades, perceptions of atherosclerosis’ pathophysiology morphed considerably. We once imagined atherosclerosis as a lipid storage disease in need of mechanical artery reopening but now recognize it to be a multifactorial condition largely driven by an overactive and dysfunctional immune system.1–4 Some therapeutic goals have been met, leading, for instance, to reduced acute myocardial infarction incidence and mortality.5 Another major success was the identification and partial elimination of smoking as a risk factor. However, atherosclerosis still leads the world’s mortality statistics.6 The life expectancy of atherosclerosis patients may have changed, but their numbers are legion, despite medical progress.6 Here we take a fresh look at 4 lifestyle-associated risk factors by asking how they may be linked to inflammation in the vascular wall. More specifically, we ask how psychosocial stress, diet, physical activity, and sleeping patterns influence the supply and phenotype of innate immune cells in atherosclerotic plaque. Epidemiological data confirm these 4 lifestyle-related disease modifiers as highly potent risk factors for atherosclerotic disease.7 For instance, daily fruit and vegetable consumption combined with regular physical activity associate with an odds ratio of 0.6 for myocardial infarction.7 Motivated by emerging connections between stress, diet, physical activity, sleep, and the immune system, we review data (or lack thereof) connecting lifestyle modifiers with immunity and hematopoiesis, and identify areas that require further study. Discussing these links, we propose testing several hypotheses to deepen our understanding of how lifestyle influences crosstalk between hematopoiesis, immune cells, and the cardiovascular system. Lifestyle is associated with potent preventive and anti-inflammatory effects: we argue that uncovering the involved molecular and cellular pathways could guide us toward new therapeutic targets.

Atherosclerosis Risk Factors

Epidemiological studies identify several risk factors for atherosclerosis, including age, sex, hypertension, low-density lipoprotein and high-density lipoprotein cholesterol, triglycerides, smoking, family history, obesity, diabetes mellitus, and pre-existing chronic inflammatory conditions such as rheumatoid arthritis.7,8 These risk factors vary in prevalence and potency and are often combined in patients with severe atherosclerosis, which supports the concept that atherosclerosis is a multifactorial disease.7 Identifying risk factors’ putative mechanisms of action has already led to some successful therapeutic strategies, particularly statins and antihypertensive drugs that inhibit atherosclerotic progression and reduce its...
complications by lowering low-density lipoprotein cholesterol and reducing blood pressure, respectively. These examples may serve as motivating blueprints for scrutinizing stress, diet, physical activity, and sleep habits as potential modifiers of immunity, hematopoiesis, and ultimately cardiovascular disease.

Role of Macrophages in Atherosclerotic Plaque

Macrophages are innate immune cells that reside in large numbers in all major healthy and diseased tissues. Named for one of their functions, phagocytosis of pathogens and other foreign bodies, macrophages’ numerous specific tasks depend on their lineage, host tissue, and phenotype. Macrophages’ tissue-specific functions are diverse, spanning bone resorption, iron recycling, antigen presentation, temperature regulation, instruction of hematopoiesis and tissue-regenerating progenitors, modulation of neural synaptic activity, among others. Recent work highlights the abundance and importance of tissue-resident macrophages, which also inhabit the healthy vascular wall and myocardium. Although resident macrophages in the steady-state vascular wall and plaque macrophages belong to the same class of innate immune cells, they may be functionally distinct. In the diseased vessel wall, macrophages accumulate progressively and fail to remove intimal cholesterol deposits. Instead, macrophages assume tissue destructive inflammatory phenotypes, resembling what has been termed M1 polarization in vitro. Macrophages may give rise to foam cells and die locally, forming the necrotic cores frequently found in ruptured plaques. While in plaque, inflammatory macrophages interact with other immune and stromal cells and secrete proinflammatory molecules that lead to tissue destruction. The cells are important sources for proteases that destabilize the arterial wall by digesting extracellular matrix, contributing to thinning of the fibrous cap and rendering lesions more vulnerable to rupture. It is clear that macrophages are central to atherogenesis and its ischemic complications; however, we are still far from therapeutically harnessing the triggers that induce the drastic atherosclerosis-associated changes in arterial macrophage number, phenotype, and function linked to myocardial infarction and stroke.

Macrophage Lineage

Although recent reports suggest that macrophage-like cells in plaque may also derive from smooth muscle cells, the bulk of data support the idea that lesional macrophages derive from genuine hematopoietic progenitors residing in the bone marrow and spleen. The life span of plaque macrophages is rather short: bromodeoxyuridine tracking experiments in 4-month-old apolipoprotein E-deficient (Apoe) mice illustrate that the entire plaque macrophage population renews within 1 month and consequently relies on newly produced cells. Depending on disease stage, macrophage production is a function of monocyte recruitment from circulation and local plaque macrophage proliferation, whereas reduced recruitment, along with macrophage death and exit from plaque likely contributes to lower macrophage numbers in the arterial wall. Below we discuss current knowledge on factors that regulate monocyte and macrophage supply in the inflamed vessel wall.

Bone Marrow Hematopoiesis

After birth, bone marrow is the primary site of hematopoiesis, giving rise to red blood cells, platelets, and circulating leukocytes. A major theory posits that the hematopoietic system is hierarchical, with pluripotent hematopoietic stem cells (HSCs) residing upstream in the differentiation cascade, giving rise to progenitors with progressively decreased renewal capacity but increased specificity for particular leukocytes. HSCs are rare (1 in 10000 bone marrow cells) and relatively quiescent (<5% are in cell cycle; Figure 1). Hematopoietic progenitor quiescence and the marrow’s staggering output are delicately balanced by extrinsic components produced by niche cells collaborating with cell-intrinsic regulatory machinery. Several stromal cells, including endothelial cells, osteoblasts, macrophages, and mesenchymal cells, secrete messengers that bind to receptors on hematopoietic cells to regulate their proliferation, migration, and lineage bias (eg, M-CSF, GM-CSF, CXCL12 also known as stromal cell derived factor 1, and stem cell factor; Figure 2). Hematopoietic progenitors may also directly sense circulating danger signals, such as toll-like receptor ligands in the setting of infection or β-adrenergic transmitters such as norepinephrine. In addition, adhesion molecules including vascular cell adhesion protein-1 and E-selectin retain HSCs in specialized hematopoietic niches. These extrinsic signals translate into HSC activity via various transcription factors. Among them, several are known to induce HSC bias toward myeloid cell production, including PU.1, C/EBPα, Egr-1, Irf8, KIf4, and Mafb. Once monocytes and neutrophils are produced, their release into circulation is tightly regulated by several signals, including CCR2 ligands and CXCL12. In addition, blood monocyte numbers oscillate according to circadian rhythms by as much as 100%, with a diurnal peak and nocturnal nadir (in humans).

Despite our growing understanding of macrophages’ importance in atherosclerosis and blood monocyte levels’ impressive correlation with cardiovascular mortality, less is known about how monocyte production changes in cardiovascular disease. During atherosclerosis, monocyte levels increase progressively and more abruptly after acute myocardial infarction, which implies that acceleration of myelopoiesis associates with disease progression. Impaired cholesterol handling directly increases hematopoietic progenitor activity, and ischemic heart disease alters many bone marrow regulation signals, including inflammatory molecules such as interleukin (IL)-1β, toll-like receptor ligands, and chemokines. In addition, altered sympathetic activity, as

Nonstandard Abbreviations and Acronyms

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<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>Apoe</td>
<td>apolipoprotein E</td>
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<tr>
<td>Cxcl</td>
<td>C-X-C chemokine ligand</td>
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<td>GM-CSF</td>
<td>granulocyte macrophage colony-stimulating factor</td>
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<td>HSC</td>
<td>hematopoietic stem cell</td>
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<td>HSPC</td>
<td>hematopoietic stem and progenitor cell</td>
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<td>IL</td>
<td>interleukin</td>
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<td>OSAS</td>
<td>obstructive sleep apnea syndrome</td>
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<td>TNF</td>
<td>tumor necrosis factor</td>
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observed after myocardial infarction, substantially changes the hematopoietic bone marrow niche. However, we are only beginning to understand how cardiovascular risk factors and lifestyle may affect hematopoietic activity and location.

**Splenic Monocyte Supply**

Although the bone marrow is the primary monocyte production site in the steady state, other lymphoid organs, such as the spleen, can produce monocytes in inflammatory conditions. During atherosclerosis, hematopoietic stem and progenitor cells (HSPCs) mobilize from the bone marrow in large numbers and, instead of returning to the bone marrow, settle in the spleen’s red pulp, where they proliferate and differentiate, giving rise predominantly to neutrophils and monocytes. This shift of myeloid cell production from the bone marrow to the spleen, we hypothesize, may be a major factor in the systemic oversupply of inflammatory monocytes and neutrophils. We still know little about the mechanisms that drive this shift from medullary (ie, in bone marrow) toward extramedullary hematopoiesis, but some thought-provoking clues are emerging. In response to high-fat diet, increased mobilization from the bone marrow depends on IL-23 production by lipid-rich splenic macrophages and dendritic cells. According to the model, IL-23 activates a cytokine cascade that culminates in increased G-CSF, a known mobilizing factor in the bone marrow. Once HSPC seed the spleen, they must anchor to the splenic parenchyma to proliferate and differentiate. The mechanisms orchestrating HSPC retention in extramedullary sites remain poorly understood but may involve S1P signaling. Once retained, HSPCs proliferate and give rise to monocytes and neutrophils. Factors intrinsic to HSPC, such as augmented expression of the β common chain, the ligand for the growth factors GM-CSF, IL-3, and IL-5, promote HSPC proliferation. Likewise, factors extrinsic to HSPC may be relevant. In response to infection and atherosclerosis, a GM-CSF–producing population of B cells, the innate response activator B cells, develops in the spleen and may instigate heightened myeloid cell production in the organ. Finally, spleen-produced monocytes and neutrophils mobilize, enter the circulation, and infiltrate atherosclerotic lesions.

Many questions about spleen-derived monocytes’ and neutrophils’ importance to the overall myeloid response remain unanswered. It is unclear, for example, whether splenic monocytes are identical to their bone marrow–derived counterparts or whether they carry a generative signature that endows them with differential function. Are splenic monocytes a source of inflammatory (M1-like) plaque macrophages or do they give rise to cells with an alternative resolution phenotype? To test these ideas, careful fate-mapping studies are necessary. Because the spleen is dispensable to life, it is tempting to tackle these questions with splenectomy. However, the surgical procedure removes an organ that is nevertheless important to many immunologic processes. For example, removing the spleen also removes a large supply of B cells, which play vital roles in innate and adaptive immunity, not to mention macrophages known to scavenge senescent erythrocytes and, likely, monocytes and neutrophils. Thus, removing the spleen probably obscures the true contribution of the organ’s myeloid cell production.
During atherosclerosis, monocytes continuously migrate to the vessel wall, where they can become lesional macrophages. Monocyte recruitment depends on chemokine-mediated migration, which is essential to the development of atherosclerosis. But monocyte recruitment is not the only process that dictates macrophage accumulation in atherosclerosis. During the years, several studies have argued that lesional macrophages renew locally and augment their numbers through proliferation, that is, cell division within the plaque. This concept did not readily catch on, partly because it was unclear whether proliferation was important and whether the proliferating cells were indeed macrophages. More conclusive recent evidence supporting macrophage proliferation in various contexts and tissues provided an impetus to determine the relative contributions of monocyte recruitment and macrophage proliferation in atherosclerosis. Fate mapping and parabiosis studies have shown that macrophage proliferation is key to macrophage lesional accumulation, especially in established atherosclerosis. To be sure, macrophages are not stem cells and probably divide no more than a few times before dying or exiting the lesion. Moreover, these studies do not negate the contribution of monocyte recruitment, and indeed, locally proliferating macrophages derive from recruited monocytes; because all macrophages can eventually be replaced with bone marrow cells argues against the idea that proliferating macrophages originate from smooth muscle cells. Rather, the studies show the nuance of...
the spatiotemporal dynamics of macrophage accumulation, while reconciling reports that argue against monocyte recruit-
ment as the ultimate process dictating macrophage number. A
revised model proposes that lesions grow through the coor-
dinated steps of monocyte production, recruitment, and local
macrophage proliferation.53–55 It remains unclear how locally
sourced macrophages differ from macrophages that directly
derive from circulating monocytes, although recent studies
indicate that the local environment is a crucial determinant of
eventual function.56–60

Hematopoietic Tree on the Move: Macrophage
Production Shifts to Periphery in Atherosclerosis
In steady-state and healthy individuals, hematopoiesis occurs
in the bone marrow.61 Migration of hematopoietic progeni-
tor cells is limited, as only a few hundred are found outside
the marrow.33 This rather tight control loosens during the
chronic inflammation of atherosclerosis: progenitor cells
now travel through the periphery in greater numbers and seed
the spleen, which contributes to increased leukocyte produc-
tion.30 Monocytes made in bone marrow and spleen circulate, extrav-
atasate, and then give rise to proliferating plaque macrophages.
We speculate that this shift in hematopoietic topography from
the bone marrow toward the spleen and atherosclerotic plaque
promotes disease progression (Figure 3). Comparing the cells
produced outside the bone marrow with those that arose inside
will reveal whether peripherally sourced cells (ie, via extra-
medullary hematopoiesis) are more or less inflammatory than
centrally produced cells (ie, via medullary hematopoiesis) and
whether the 2 cells types have similar phenotypes. Currently,
we do not fully understand what loosens the marrow’s control
and why myelopoiesis shifts to peripheral sites as atheroscle-
rosis progresses. Uncovering these mechanisms may allow us
to design interventions that tighten central control and contain
hematopoiesis in the bone marrow to reduce plaque inflamma-
tion. We hypothesize that atherosclerosis risk factors, and the
lifestyle modifiers discussed below, initiate the macrophage
production shift to peripheral sites by promoting HSC and
progenitor release from the bone marrow (Figure 3).

Healthy Versus Disease-Promoting Lifestyle
Diet/Obesity
Mice lacking crucial reverse cholesterol transport compo-
nents, such as ApoE or the low-density lipoprotein receptor,
develop atherosclerosis while consuming a so-called Western
diet. These artificial models are useful because the mice devel-
op lesions similar to those that develop in humans. In contrast,
wild-type mice (ie, with intact reverse cholesterol transport)
fed a high-fat diet do not develop atherosclerosis, but they gain
weight and become obese with age. Elaborating the metabolic
changes that occur during obesity and the links between obe-
sity and cardiovascular disease are beyond the scope of this re-
view and have been extensively discussed elsewhere. Indeed,
research exploring the links between obesity, metabolism, and
inflammation has flourished, and we guide the reader to recent
reviews.62–66 However, we would like to focus our discussion
on a few insights that are particularly relevant to how diet/obe-
sity may affect hematopoiesis. A landmark observation, made
>10 years ago, demonstrated that macrophages accumulate in
adipose tissue.67 By showing the existence of macrophages in
fat, the study proved to be immensely influential as it was
evidence for the concept that adipocytes, metabolism, and
immunity are functionally linked. Four years later, a second
paper revealed that macrophages residing in obese mice dif-
ered phenotypically from those in lean mice.68 The study was
particularly interesting because the data suggested, by virtue
of focusing on CCR2, the chemokine receptor for CCL2, that
macrophages accumulating in fat tissue are monocyte derived.
The idea was linked conceptually with 2 independent athero-
sclerosis studies, published in the same issue, demonstrating
that inflammatory CCR2+ Ly-6Chigh monocytes accumulate in
atherosclerotic lesions and give rise to macrophages.23,38 More
studies on the origin and function of adipose tissue resident
macrophages followed.63–66 One study of particular interest
to our discussion concluded that adipose tissue macrophages
promote monocyte production by secreting IL-1β.69 The data
implied that a vicious cycle operates in obesity whereby adi-
pose tissue macrophages beget more macrophages by releas-
ing endocrine factors that induce the generation of monocytes
in the bone marrow remotely. This increase in monocyte
production, triggered by obesity, may also enlarge the sys-
temic monocyte pool available to recruitment to other sites,
especially atherosclerotic plaque. This argument provides a
hypothetical connection between the lifestyle factor, obesity,
hematopoiesis, and atherosclerosis progression that is inde-
pendent of direct cholesterol deposition in the vascular wall.
A second study focusing on the toll-like receptor adaptor Myd88
has deepened the connection between adipose tissue and the
periphery, demonstrating that Myd88 fosters an inflammatory
(M1) macrophage phenotype in adipose tissue and associates
with systemic inflammation.70 These studies are highlights
that by no means represent the entire breadth of work on the
subject. Nevertheless, both converge on the idea that obesity
is an inflammatory disease that stimulates the production of
monocytes, their recruitment to adipose tissue, and differen-
tiation to inflammatory macrophages. In this context, then, it
is tempting to speculate that obesity alters macrophage supply
(ie, the hematopoietic tree) in ways that resemble disturbances
observed in atherosclerosis. Perhaps the links between obesity
and atherosclerosis owe more to altered hematopoiesis than
has been appreciated to date.

Another area of study with possible connections between
diet, atherosclerosis, and hematopoiesis focuses on the micro-
biome. We are learning that diets rich in particular nutrients
predispose to atherosclerosis by influencing the gut intestinal
flora.71 Studies on allergic airway inflammation have shown
that dietary fiber content alters the composition of the gut and
lung microbiota, which changes the concentration of short-
chain fatty acids in the blood, thus altering hematopoiesis in
the bone marrow.72 Although these connections are still tenu-
os, they challenge us to reorient our understanding of ather-
sclerosis to include specific dietary ingredients and think more
deeply about dietary inputs and their influence on disease.

Psychosocial Stress
Chronic stress is a recognized risk factor for athero-
sclerosis.73,74 Epidemiology data show increased risk of
cardiovascular events on both acute and chronic time scales. Earth quakes\(^5\) and the World Cup soccer championship\(^6\) increase the incidence of acute myocardial infarction, while post-traumatic stress disorder, for instance in combat veterans,\(^7\) confers increased long-term risk of atherosclerotic events. Permanent stress at work or home results in an odds ratio of 2.14 and 2.12, respectively, for myocardial infarction.\(^9\) The manifold effects of stress on immunity are only partially understood.\(^8\) Systemic release of stress hormones triggers organismal readiness for fight and flight. Immune modulation during and after exposure to stress prepares for injuries or infections that may arise from dangerous, stressful situations. Exposure to stress activates 2 major pathways: the limbic-hypothalamic-pituitary-adrenal axis, which leads to systemic release of corticosteroids,\(^9\) and the sympathetic nervous system. In particular, cortisol has numerous immunosuppressive effects on leukocytes, which express the glucocorticoid receptor to varying degrees (www.immgen.org). When hypothalamic fear centers activate the sympathetic nervous system, increased levels of systemic catecholamines signal through adrenergic receptors expressed by stromal and immune cells.\(^10\) A high autonomous tone may increase cardiac output, vasoconstriction, and blood pressure, and thus contribute to cardiovascular risk.

Leukocytes and hematopoietic progenitors express \(\alpha\)- and \(\beta\)-adrenergic receptors. For instance, bone marrow hematopoietic progenitors\(^11\) and splenic red pulp macrophages (www.immgen.org) express \(\beta_2\)-adrenergic receptors. Thus, the neurotransmitter and stress hormone norepinephrine may increase inflammatory activity of macrophages and their progenitors. In the bone marrow, norepinephrine acts via \(\beta_3\)-adrenergic receptors expressed on stromal cells that are part of the hematopoietic niche.\(^12\) Sympathetic nerve fibers run along bone marrow arterioles and release norepinephrine which then acts locally on mesenchymal cells (Figure 2). These cells are a source of CXCL12, a cytokine that retains hematopoietic progenitors and neutrophils within the marrow.\(^13\) CXCL12 also promotes HSC quiescence. When bone marrow levels of norepinephrine increase, CXCL12 levels decline, pushing HSCs into active phases of the cell cycle, likely enhancing their migratory capacities. Sympathetic tone’s relationship to CXCL12 levels and HSC activity was first described in the setting of circadian rhythms.\(^14\) The central clock located in the suprachiasmatic nuclei receives retinal cues synchronizing dark/light cycles with circadian activity of the autonomous nervous system, which then confers these rhythms to the hematopoietic marrow.\(^15\) Sympathetic activity after acute myocardial infarction in mice also activates HSC migration, leading to extramedullary monocyte production and aggravating atherosclerosis in ApoE\(^{-/-}\) mice.\(^16\) Ischemic stroke in mice similarly triggers bone marrow activation.\(^17\)

In mice, psychosocial stress leads to chronically increased autonomous tone in the bone marrow.\(^18\) When mice were exposed to variable stressors such as crowding, isolation, cage tilt, and changes in bedding during a time period of 3 to 6 weeks, norepinephrine levels rose in their femoral marrow, thereby modulating the hematopoietic microenvironment. As described for circadian signaling, the neurotransmitter acted through \(\beta_3\)-adrenergic receptors on niche cells, reducing CXCL12 levels in stressed mice. In return, the percentage of cycling hematopoietic stem and progenitors increased, as did bone marrow production and release of monocytes and neutrophils. Consequently, their numbers in circulation rose. Mice that genetically lack the \(\beta_3\)-adrenergic receptor exhibited a blunted response to stress, as their CXCL12 levels and bone marrow leukocyte output were unchanged.\(^19\) These data correlate well with reports of increased myeloid cell production after stress exposure\(^20\) and injection of epinephrine into mice.\(^21\) ApoE\(^{-/-}\) mice exposed to stress develop a more inflamed plaque phenotype, with higher macrophage numbers and increased protease activity,\(^22\) reminiscent of vulnerable plaques in humans. Systemic effects of noradrenaline may be milder but also increase lesion size in some regions of the aorta in ApoE\(^{-/-}\) mice.\(^23\) The effects of stress on splenic myelopoiesis and plaque macrophage proliferation are currently completely unknown. Although the arterial vasculature is richly innervated by sympathetic fibers, making local autonomous nervous system effects on plaque likely, the spleen seems to lack sympathetic innervation. In contrast, the vagus nerve readily interacts with splenic leukocytes, dampening inflammatory responses.\(^24\)

In humans, acute and chronic stress exposure increases blood leukocytes, likely through demargination and possibly also through increased cell production.\(^25\) Furthermore, leukocyte inflammatory gene expression is influenced by stress levels.\(^26\) It is tempting to speculate that the stress-related increase in leukocytosis and inflammation promotes atherosclerotic progression and complication, as blood monocytosis predicts cardiovascular mortality.\(^27\) This hypothetical disease mechanism, especially the link to bone marrow activity, should be investigated further in human patients.

Sleep Disorders

We are, or should be, asleep for at least one third of our lives. Sleep is essential to life; its acute deprivation reduces alertness and performance, impairing memory and cognition, whereas chronic sleep deprivation is associated with high blood pressure, stroke, myocardial infarction, and heart failure. Numerous causes of sleep deprivation range from self-imposed denial to stress-related insomnia, sleep apnea, and mental illness. Despite the many connections between sleep deprivation and disease, we still know little about the underlying biology and connections to disease. In light of this discussion, is there any evidence that sleep predisposes to heart disease by affecting hematopoiesis?

Arguably the most work devoted to understanding the effects of sleep deprivation has been done in the context of sleep apnea, a condition that affects 2% to 5% of the general population, is more prevalent in obesity, and is a risk factor for cardiovascular disease. Sleep apnea is characterized by repeated stops and starts in breathing.\(^28\)\(^,\)\(^29\) Physiologically, the disturbance results from the collapse of the upper airway during sleep, and patients repeatedly wake up so as not to asphyxiate. Complications arising from sleep apnea, such as high blood pressure, atrial fibrillation, or congestive heart failure, likely result from sudden drops in blood oxygen. Because of this, it might be difficult to distinguish which effects are related to the multiple hypoxic events and which to sleep fragmentation.
that necessarily occurs during waking. Regardless of the primary cause, obstructive sleep apnea syndrome (OSAS) consistently correlates systemic inflammation with hypoxia and sleep fragmentation. Patients have elevated plasma levels of TNF-α, IL-6, macrophage inhibitory factor, and C-reactive protein compared with obese or healthy individuals. Moreover, patients with moderate-to-severe OSAS contain monocytes that are more adherent to the endothelium, producing more TNF-α, and more neutrophils that release superoxide. Additional evidence of how OSAS might promote atherosclerosis centers on vascular smooth muscle activation, increased lipid loading in macrophages, lipid peroxidation, and endothelial dysfunction. Although no definitive study evaluated whether OSAS associates with elevated or disturbed hematopoiesis, the connection with myeloid cells renders the link plausible and worthy of investigation.

Animal studies have attempted to show how sleep fragmentation induces inflammation in the absence of hypoxia, the potential confounder in OSAS. Animals that are prevented from falling into deep rapid eye movement sleep produce TNF-α, which associates with cognitive dysfunction induced by sleep fragmentation. Using TNF-α receptor–deficient mice, as well as neutralizing antibodies to TNF-α, it was shown that excessive sleepiness incurred by sleep fragmentation may derive from activation of TNF-α–dependent inflammatory pathways. The hypothetical connections between sleep deprivation, hematopoiesis, and atherosclerosis are further strengthened when considering circadian rhythms. The central circadian clock, located in the suprachiasmatic nuclei, allows organisms to coordinate their behavior and biology with daily and seasonal fluctuations in the day–night cycle. In the brain, CLOCK genes control transcription of a large portion of the genome. In addition, circadian fluctuations are controlled by peripheral clocks located in cells throughout the body. It is estimated, for example, that >8% of the macrophage transcriptome oscillates according to the day–night cycle, and peripheral clock genes such as Bmal1 dramatically alter monocyte numbers in the circulation. Mice exposed to dim light, which disturbs circadian rhythms, have exaggerated inflammatory responses and gain weight. Moreover, the onset of acute myocardial infarction follows a circadian pattern. It is therefore tempting to speculate that peripheral clock genes, which regulate macrophage function independently of the central clocks located in the suprachiasmatic nuclei, likewise regulate hematopoietic output and location of the hematopoietic progenitors. The study of sleep is mostly uncharted territory, and the recent identification of convective fluxes of interstitial fluid that clear β-amyloids from the brain during sleep only underscores how little we know. However, it is clear that hematopoiesis, monocyte production, and migration follow nervous inputs, and thus a connection between myelopoiesis and sleeping habits should be studied experimentally.

Physical Activity and Exercise
Physical activity, or lack thereof, greatly influences the immune system and atherosclerosis risk. Exercise is thought to lower cardiovascular risk by improving metabolic balance and consequently counteracting development of obesity. However, physical activity may also directly affect the immune system independent of metabolic pathways. For instance, the acute effects observed immediately after strenuous exercise are likely independent of chronically increased energy expenditure. In humans and mice, exercise can increase inflammatory cytokine levels (eg, TNF-α and IL-1β, IL-6, among others), leukocytosis, and trigger release of bone marrow cells into the bloodstream. Specifically, the number of circulating neutrophils, monocytes, and lymphocytes rises during exercise. Systemic sympathetic activity, which increases during exercise, is a candidate trigger for the observed increase in circulating leukocytes and progenitor cells. Interestingly, injection of epinephrine into humans cause similar changes in blood leukocyte levels as exercise does. Analogous to stress exposure, acute myocardial infarction, stroke, and during circadian regulation of hematopoiesis, exercise may acutely elevate sympathetic tone in the bone marrow and lower bone marrow CXCL12 signaling. Decrease of this chemokine may then lead to stem and myeloid cell release into blood and contribute, in addition to demargination of cells, to blood leukocytosis acutely after strenuous physical activity. The hypothesis that exercise triggers bone marrow release of leukocytes should be tested experimentally, in conjunction with exploring key hematopoietic niche factors after exercise.

During longer time frames, intense exercise is thought to suppress immunity in mice and humans, although the precise mechanisms are unclear. For instance, 12 weeks of aerobic and resistance training reduced circulating inflammatory monocytes in humans. Exercise may reduce the number of circulating lymphocytes and natural killer cells, potentially contributing to immunosuppressive effects.

The available human and rodent data on exercise’s influence on the hematopoietic system are mostly limited to acute effects showing increased bone marrow release of leukocytes and hematopoietic progenitors. Regarding chronic effects on leukocyte production by bone marrow, data are currently limited to a few insightful reports obtained in mice exposed to forced exercise. Running mice show increased bone marrow proliferation of lineage–Sca-1+ c-Kit– hematopoietic progenitors leading to increased lineage–Sca-1+ c-Kit– frequencies therein. Bone marrow transplantation into irradiated recipients resulted in more splenic colonies when bone marrow donors were subjected to forced running. Blood reconstitution was similar between sedentary and exercising bone marrow donors, suggesting a comparable efficiency of transplantation. Rigorously enumerating long-term HSC in exercising mice requires a limiting dilution stem cell assay, which has not been reported thus far. In addition, we caution that experiments described above used mild electric shocks to encourage mice to run. Although this study design ensures homogenous and well-defined physical activity, it may also confer emotional stress through administration of electric shocks. The reported data may thus reflect combined exposure to physical activity and psychosocial stress. We conclude that hematopoiesis, HSC number, and function should also be studied in voluntary running mice.

Regular aerobic exercise increases systemic vagal tone. Increased vagus nerve activity is immunosuppressive, for
instance through signaling to T cells and macrophages in the spleen.\textsuperscript{123} It remains unclear whether vagal activity affects splenic hematopoiesis, possibly through modulated macrophage activity. This is a reasonable hypothesis, as CD169\textsuperscript{+} macrophages are important contributors to the hematopoietic niche\textsuperscript{124} and because splenic macrophages express acetylcholine receptors. The hypothesis that exercise-related increase in vagal activity may reduce inflammatory macrophage activation, and thus confer protective effects on atherosclerosis, should be tested experimentally.

There are no human data on exercise’s influence on bone marrow hematopoiesis, and physical activity’s effects on many of the typical niche factors that regulate HSC proliferation have not been explored. Future studies should also verify that exercise indeed increases bone marrow HSC activity. If this was the case, one wonders how to reconcile increased HSC proliferation, which should lead to systemically increased levels of leukocytes, with the reported immunosuppression induced by strenuous exercise\textsuperscript{125} and the protective effects of exercise against cardiovascular disease. More specifically, what happens to the bone marrow of voluntary running Apoe\textsuperscript{−/−} mice, which have increased HSC proliferation because of compromised reverse cholesterol transport,\textsuperscript{25} even when they are sedentary, as all mice housed in the small cages of current research facilities could be considered sedentary? Does the liberation of HSC after bouts of strenuous exercise accelerate extramedullary hematopoiesis in the spleen? Alternatively, does exercise reduce macrophage supply, as suggested by decreased plaque protease activity measured in this setting?\textsuperscript{1026} Does systemic HSC proliferation decrease if atherosclerotic individuals exercise, because an increased vagal tone dampens splenic myelopoiesis? Is the level of exercise important, as some authors suggested a U-shaped relationship between exercise and immune function,\textsuperscript{115} similar to what has been described for alcohol consumption and cardiovascular mortality?\textsuperscript{9127} Addressing these questions experimentally will further our understanding of the hematopoietic system’s overall role in the progression of cardiovascular disease.

Conclusions

Decades of meticuloous epidemiological research provides us with rich information on atherosclerosis progression’s association with various risk factors. Some risk factors’ causal pathways were identified and harnessed therapeutically. Because atherosclerosis is far from being cured, we ought to take a closer look at lesser-understood risk factors and explore whether their pathways of action are neural, inflammatory, metabolic, or a combination thereof. We already adhere to this concept when feeding mice a high-fat diet, one of the lifestyle factors discussed above. Emerging data on the immune and hematopoietic systems suggest that both may have sizable roles in promoting cardiovascular disease. This is an opportune time to explore causalities linking lifestyle, hematopoiesis, and atherosclerosis, because studying lifestyle factors and their influence on plaque macrophage supply and phenotype may reveal unknown disease pathways amenable to therapeutic interventions. For instance, if disease-propagating lifestyle factors such as stress, sleep disorders, and unhealthy diet converge on a specific pathway that accelerates atherosclerosis, whereas positive modifiers such as physical activity have an opposing effect, this pathway should be explored as a therapeutic target to reduce macrophage supply to atherosclerotic lesions. A merger of several research fields would expedite this work, as the discussed pathways may span multiple systems, including the nervous, immune, cardiovascular, metabolic, and hematopoietic. Scientific exchange among experts in these diverse areas would better enable tracking disease mechanisms that ignore the traditional—and somewhat artificial—borders between historically grown research fields. Interdisciplinary training, funding opportunities, and research environments that encourage cross fertilization among seemingly disparate disciplines would likely accelerate this progress.

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Disclosures

None.

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B cells protect against microbial sepsis. 


Lifestyle Effects on Hematopoiesis and Atherosclerosis
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