Issues in Toxicology

Edited by Aruni Bhatnagar

Environmental Cardiology

Pollution and Heart Disease

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Environmental Cardiology
Pollution and Heart Disease

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Preface

This monograph was assembled to bring together recent developments in the emerging field of environmental cardiology. This new area of research encompasses the study of various environmental factors and their role in the genesis, severity and incidence of heart disease. Although it is widely recognized that environmental factors such as smoking, diet, exercise, and socio-economic status profoundly affect the risk of cardiovascular disease, recent work showing the effects of other environmental factors provides a more complete assessment of the depth and the breadth with which the environment affects heart disease.

This comprehensive view has emerged from three recent developments. First, there has been a relatively sudden explosion in the prevalence of diabetes and obesity, which indicates a strong environmental component. In addition, there has been an accumulation of new evidence suggesting that most cases of heart disease and diabetes could be prevented by healthy lifestyle choices. Finally, extensive studies have shown that exposure to environmental pollutants has a significant effect on heart-disease risk. Among these developments, studies in the area of air pollution provide a more detailed description of how the environment affects heart disease. These studies reveal that cardiovascular tissues are exquisitely sensitive to changes in the external environment, and they broaden the view that cardiovascular health is inextricably linked with natural, social and personal environments. Accordingly, this monograph is devoted primarily to a discussion of pollution and heart disease.

In an attempt to develop a more complete view of the environmental basis of heart disease, assessments of the cardiovascular disease burden of pollutant exposure provide an important missing piece of the puzzle. Putting this piece together with other known environmental effects allows us to see uninterrupted connections between different aspects of the environment and how together they create conditions that promote and sustain heart disease. Studies in particulate

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air-pollution research reveal a new "risk factor" for heart disease; but more importantly, they provide a new paradigm for understanding how the environment continuously affects the development of heart disease and how environmental changes adruptly trigger adverse cardiovascular events. Exposure to particulate air pollution is associated with an exacerbation of hypertension and insulin resistance, acceleration of atherogenesis, as well as plaque rupture leading to myocardial infarction. These associations suggest that environmental exposures affect all stages in the development of heart disease. Other environmental factors exert similar effects. Hence, an understanding of environmental influences is likely to be important, not only in the prevention of heart disease, but in its treatment and management as well.

The introductory chapter provides a general view of the field and outlines the effects of different aspects of the environment on heart disease. It provides a context for the discussion that follows, and it maps pollution research within the overall topography of environmental cardiology. Chapter 2 gives an overview of the cardiovascular effects of particulate matter, and Chapter 3 discusses the epidemiological studies supporting this link. In subsequent chapters, the effects of pollution on different aspects of cardiovascular disease – hypertension, stroke, heart failure, ischemic heart disease and atherogenesis – are presented. Because of a close association between diabetes and heart disease, a discussion of the effects of particulate matter on diabetes is included in Chapter 5. Later chapters discuss the effects of individual pollutants such as vehicular emission, metals and aldehydes. A review on manufactured nanoparticles is included because these particles represent an important new threat to cardiovascular health.

Although not exhaustive, this collection provides an inclusive view of research in this area. Like all areas of active investigation, this is a work in progress and therefore subject to modification, elaboration or even revision by future discoveries. Research in this area is progressing at a rapid pace, and therefore it is important to pause and survey how far we have come and to consider where we should go from here. To this aim, the monograph brings together for the first time a broad discussion on the role of the most important environmental factors that affect heart disease.

Many of the studies discussed here suggest that a significant burden of heart disease could be lifted by removing unhealthy environmental influences. These studies show that, for the most part, heart disease does not develop in healthy, unpolluted environments or in individuals who make optimal lifestyle choices and are in synchrony with the primordial rhythms of their natural environment. In addition, it has been shown that the risk of heart disease is rapidly and robustly affected by changes in the environment. Collectively, these facts imply that there is a causative link between the environment and heart disease. While the disease manifests in the individual, its origins frequently lie in the environment. Attributing heart disease to unhealthy environments, however, does not invalidate or deny the role of genetic susceptibility. Genetic and metabolic factors are undeniably important formal and material causes of heart disease. They regulate the forms, the manifestation and the severity of

heart disease. But, the environment is usually the efficient cause, as it often engenders the right conditions for the development of heart disease, and in doing so it acts as a primary trigger to which genetic and metabolic processes respond.

While current therapies are aimed at treating pathological responses (blood pressure, cholesterol levels) in the individual, less emphasis is placed on controlling or extinguishing the environmental triggers that elicit these responses. In this regard, the understanding that emerges from this monograph suggests that we must be more alert to the effects of the environment and develop strategies that target not only the diseased individual but the unhealthy, disease-causing environment as well. Because heart disease arises mostly from unhealthy environments, targeting the environment is likely to provide more tangible gains. Although much work is still required to fully redeem the promise of this vision, the research presented here could facilitate and stimulate new investgations and, thereby, encourage the development of a more coherent view of environmental cardiology.

In the last few years, our understanding of the environmental factors that contribute to the risk of heart disease increased significantly. The most rapid growth has been in the area of air-pollution research. This area has attracted wide attention and has been a topic of several commentaries, reviews and symposia. It has also been the subject of a recently updated scientific statement from the American Heart Association. Nevertheless, this monograph fills an important void. It is the first attempt to provide a comprehensive account of the effects of pollutants on heart disease and to integrate this area of research within the overall theme of environmental cardiology. Thus, the publication of this monograph is an important milestone in the development of this field, and the book itself is likely to serve as a valuable resource for both new and established investigators interested in this area of research. The overview and perspectives, as well as the detailed discussions on individual issues, may prove helpful to students and trainees on their path to new discoveries.

The most important element in discovery, however, is the discoverer. All that we know about the environment and its effects on heart disease comes from the work of several creative and committed investigators to whom we remain indebted. In particular, I am thankful to the extraordinary league of scientists who have made key discoveries in this area and who have contributed to this monograph. Their relentless pursuit of truth, even when its path may not be clear or fashionable, is inspirational. I am both proud and humbled to be their colleague and to be able to participate in the discussion they started. I am grateful for the time they took from their hectic research schedules to contribute to this book, and I am convinced that their work will continue to inspire the next generation of scientists.

On behalf of my colleagues, I also wish to express gratitude to the enlightened leadership at the National Institutes of Environmental Health Sciences and the Environmental Protection Agency. They are equal partners on this journey, and their support has been instrumental in the development of this

field. Finally, I would be remiss if I did not acknowledge my deep appreciation for members of my family. They have suffered my long absences with extraordinary patience and understanding. But always it was the return home that made it all worthwhile.

Aruni Bhatnagar

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CHAPTER 1

Environmental Basis of Cardiovascular Disease

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1.1 Introduction

The term cardiovascular disease (CVD) refers to a group of illnesses caused by the disorders of the heart, blood vessels and blood flow. The most common cause of cardiovascular diseases is atherosclerosis, which is the hardening of arteries due to the formation of an atheromatous plaque. Abrupt changes in blood flow in atherosclerotic vessels result in acute myocardial infarction and stroke, which are the major clinical manifestations of chronic changes in the vessel wall. In the heart, ischemic injury due to atherosclerotic disease often leads to arrhythmia, hypertrophy, cardiomyopathy and heart failure. Heart disease is accompanied by chronic metabolic and physiological changes that precede and contribute to its clinical manifestations. These include metabolic changes such as high cholesterol (hypercholesterolemia) and insulin resistance and physiological changes such as an increase in blood pressure (hypertension) and changes in cardiac contractility. Although the causes of diabetes are not well understood, diabetes primarily affects the heart and blood vessels and is, therefore, considered to be a major CVD risk factor. Therefore diabetes and obesity are included in this discussion of heart disease.

Significant CVD is also associated with rheumatic disease, which is due to myocardial damage caused by streptococcal bacteria and congenital malformation of the structures of the heart or blood vessels. Several other types of

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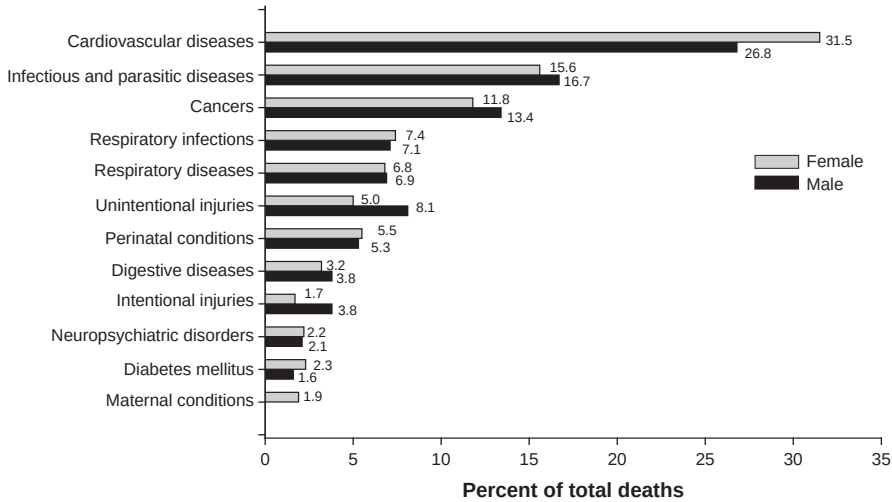

Figure 1.1 Distribution of deaths in the world in 2004 by leading cause groups and gender (WHO report).

congenital CVD are also common. These defects could be overt, resulting from gross malformation of major blood vessels or myocardial tissue in the fetus, or they may be more subtle, leading to an increase in susceptibility to stress or exercise. Congenital defects or prolonged hypertension and infectious diseases could also result in the dilation and rupture of the aorta leading to aortic aneurysm and dissection. Additionally, cardiovascular disorder associated with deep vein thrombosis and pulmonary embolism could result from blood clots in the leg veins that can dislodge and move to the heart and the lungs.

As a group, CVD is the leading cause of death world-wide (Figure 1.1). According to the WHO in 2004, CVD accounted for nearly 30% of all deaths world-wide. It killed twice as many people as infectious and parasitic disease and 3 times as many people as all forms of cancer. Globally, most (43–45%) cardiovascular deaths are due to coronary heart disease (CHD) or ischemic heart disease (IHD), whereas stroke accounts for 33% of CVD. A similar distribution of CVD deaths has been reported for countries such as the US (Figure 1.2).[1]

These statistics suggest that heart disease is the major cause of mortality world-wide. Although the prevalence of heart disease varies considerably (*vide infra*) it still remains a major cause of death in all human populations regardless of their geographic location or ethnicity. It shows no preference for gender or economic status. Both men and women appear to be equally susceptible. World-wide, more women (31.5%) than men (26.8%) die of heart disease. Even in low-income countries (per capita $\leq$\$825) IHD is the number two leading cause of death (9.4%), second only to deaths caused by lower respiratory infections (11.2%), whereas in middle and high income countries (\$10,066 or more) IHD and cerebrovascular disease account for 25 to 28% of all deaths

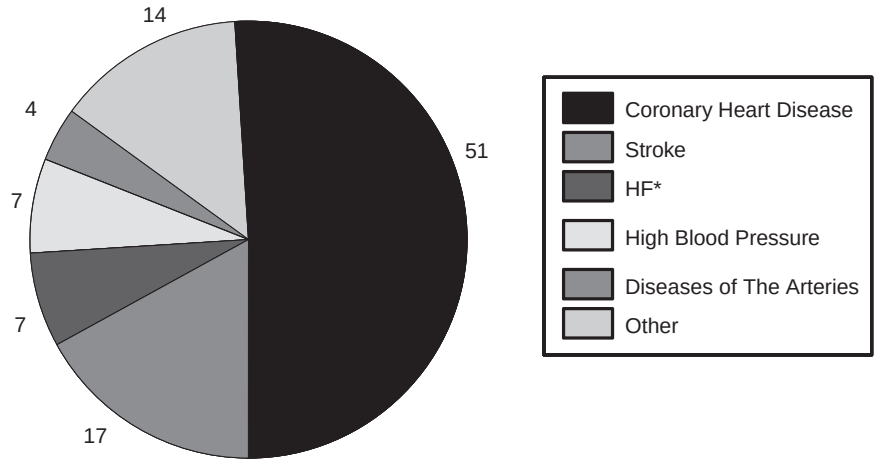

Figure 1.2 Per cent breakdown of deaths due to cardiovascular diseases in the United States in 2006. Data are derived from the 2010 report of the American Heart Association.[1] * Not a true underlying cause. The data may not add to 100% because of rounding.

(WHO, 2005). What is more alarming is that the prevalence of heart disease in increasing. The WHO estimates that 80% of all current CVD deaths are in developing, low- and middle-income countries and it is estimated that by 2010, CVD will be the leading cause of death in developing, low-income countries as well. In developed countries, the emergent epidemics of diabetes and obesity are threatening to erode the pattern of recent gains in health. In the US, the increase in obesity alone has been forecasted to slow down the increase in life expectancy that has been steadily increasing since the early 20th century.[2] Thus, CVD is the most frequent cause of death throughout the world, independent of economic status, gender, or ethnic differences.

The universally high burden of CVD and the extraordinarily high rates of CVD mortality across all communities, suggests that humans as a species are particularly prone to heart disease. It may be argued that CVD is an inevitable consequence of aging, that blood pressure and cholesterol levels inexorably increase with age and that if an individual survives middle age without succumbing to infectious disease, sporadic cancers, accidents or violence, their most likely fate is cardiovascular death. This view is consistent with data showing that the risk of dying from CVD increases with age. In the US, the percentage of population with CVD increases from 14.9 and 8.7% for men and women of 20–39 years of age to 78.8 and 84.7% for men and women more than 80 years of age. It has been suggested that because heart disease develops more often in old individuals, it is not subject to direct selective pressure, *i.e.* that natural selection during evolution is unable to weed out these diseases as they do not affect reproductive success. Natural selection, it is believed, tends to maintain the frequency of genes that increase reproductive success even if the

genes have other effects that increase disease susceptibility in older age.[3] However, as we shall see, these arguments do not take into account the important role of the environment, which affects not only the long-range evolutionary susceptibility to disease, but also the proximate causes that lead to the disease development in a specific individual. Moreover, changes in the environment can modify (slow down or accelerate) age-dependent changes in the heart and blood vessels. In addition, a changing environment could continuously alter the context within which the effects of a gene manifest. Thus, a gene could be beneficial in one environment but not the other. As a result, changes in the environment could impart maladaptive predilection to a previously well-adapted genetic variance; thereby significantly and robustly modifying disease susceptibility.

1.1.1 *My Family and Other Animals*

The high prevalence of CVD in human populations suggests shared genetic susceptibility. In comparison with other species, humans are genetically very similar. The low genetic diversity in humans has been linked to a rather small population of ancestors from which modern humans have descended. By some accounts, all modern humans are descendents of a small ancestral family of only 10,000 individuals.[4] As a result, humans are very similar to each other. Moreover, their gene pool has remained shallow because humans spread very quickly over vast expanses of land without acquiring sufficient genetic diversity. Because of their high cognitive abilities and greater capacity to adapt to different environments they migrated to different parts of the planet and segregated into small inbreeding populations, which did not have the time to diverge before significant interbreeding began again. It is therefore not surprising that all humans have similar disease susceptibility and that they succumb to very similar afflictions. But if we take a less parochial view and look outside the human family we might ask – what about other species? Are other animals susceptible to heart disease as well?

In the wild, captivity or domestication, mammals such as dogs, rabbits, rats and mice rarely develop spontaneous atherosclerosis of the type seen in humans. Even when cholesterol metabolism in mice is severely compromised by genetic engineering, they rarely suffer from myocardial infarctions or stroke. This difference may be due to the large evolutionary distance that separates humans from most other mammals and perhaps it is more instructive to look at the great apes, particularly gorillas and chimpanzees. Humans, gorillas, and chimpanzees have descended from a common ancestor that lived 7.3 million years ago.[5] The chimpanzees are our closet living cousins from whom we diverged 5.4 million years ago.[5] Nevertheless, the amino acid sequences of humans and chimpanzees show 99% homology.[6] Hence, it may be expected that, because of their high genetic similarity, humans and chimpanzees would have similar disease susceptibility and might die of similar causes.

Fortunately, several investigators have studied chimpanzee and gorilla mortality both in the wild and in captivity. As expected, the life expectancy of a

chimpanzee in the wild is shorter than in captivity. In the wild most chimpanzees live to be around 15 years of age, although occasionally 40- to 50-year-old chimpanzees have been sighted.[7] In the wild they succumb mostly to infectious diseases, most chimpanzees die of respiratory infections[8] while gorillas fall prey to various types of entrocolitis due to viral or fungal infections.[9] In captivity, however, it has been found that cardiac disease is the primary cause of mortality in both gorillas[10] and chimpanzees.[11] Cardiac disease has been reported to be responsible for 41%[11] of deaths of captive adult lowland gorillas and 67.8%[12] of captive chimpanzees. However, the type of heart disease described in chimpanzees and gorillas is not the type commonly seen in humans. In one study most of the heart disease in chimpanzees was reported to be due to an unusual form of cardiomyopathy that was associated with congestive heart failure and the presence of multifocal to coalescing areas of fibrosis, necrosis, mineralization and inflammation[12] and ventricular arrhythmias.[11] Similar findings have been reported by others.[13–15] This type of diffuse cardiac fibrosis leading to congestive heart failure has also been observed in western lowland gorillas.[16] Such pathology is rarely seen in humans and it certainly does not contribute to garden-variety heart disease that kills most humans. In humans, a majority of heart disease is due to atherosclerosis that results in coronary artery disease and stroke. Together, these diseases account for 76% of all cardiovascular deaths world-wide. In contrast, only 2.3% of the captive chimpanzees have been reported to have atherosclerotic disease and although hypertension and hyperlipidemia have been diagnosed in both captive chimpanzees[11] and gorillas,[17] these conditions were found not to be associated with coronary heart disease or with atherosclerosis.

There may be several reasons why chimpanzees are genetically less susceptible to atherosclerotic disease. One of these may relate to the 1% difference in the human and chimpanzee genome. While this does not seem like much, it accounts for the starkly different cognitive, cultural and behavioral differences between humans and chimpanzees. However, this appears not be the case because most the genetic differences between human and chimpanzees are in cortical genes. By contrast, the genes in chimpanzee hearts and livers are nearly identical to humans.[18] Thus, it seems unlikely that humans have recently acquired genes that have increased their susceptibility to metabolic diseases. An alternative explanation is that perhaps during evolution, humans have lost some of the genes that protect chimpanzees from atherosclerotic disease. Indeed, current theories of human evolution suggest that humans have evolved from chimpanzees by loss-of-function mutations (the "less-is-more" hypothesis).[19] It is believed that in many respects, humans are "degenerate apes" who have lost, among other characteristics, much of their muscle strength, hair *etc.*, or their ability to synthesize certain metabolites such as sialic acid.[5] By shedding this excessive baggage, humans have been able to evolve at a more nimble and rapid pace than chimpanzees. Hence, it is conceivable that by losing some genes and acquiring a more retrograde phenotype, humans have become more susceptible to atherosclerotic disease. It is well known that several human diseases such as cystic fibrosis, phenylketouria, and familial breast cancer are

due to loss-of-function mutations. Nevertheless, a comparison of human and chimpanzee genomes shows that humans have not lost any of the genes that regulate cardiovascular and hepatic function. On the contrary, several common genetic polymorphisms that are clearly linked to coronary disease and diabetes in humans (*e.g.*, PPARG A12P, PON1 Q192R, and ABCA1 I883M) are ancestral alleles carried not only by chimpanzees but also by out-groups such as macaque.[20] A most likely explanation is that humans and chimpanzees carry the same gene variant and that these ancestral alleles have become human-specific risk factors, not because of a loss of function, but because of a change in the environment. These alleles are natural and widely distributed in living apes. They have evolved and they have assumed the form that they do so that the apes could adapt to their environment. It is likely that they were equally beneficial to humans in their early, ape-like environment, but because the environment in which human live now has changed these genetic variations are no longer beneficial. Instead, they increase disease risk. Thus, a change in the environment has dramatically changed the survival advantage and the disease-risk associated with specific allelic variations.

1.1.2 *Peacocks in Siberia*

Why have the genes that were protective in the ancient environment become maladapted in the current environment? There are several answers to this question.[21] One explanation is that an ancestral gene, which was adaptive in an ancient environment, is no longer beneficial in the modern environment because the environment has changed drastically. In other words, it has become maladaptive in the modern environment. Ancient and modern humans live in very different environments and the gene variants that were important for survival, growth and health under those conditions may not be protective in the modern environment (A to B; Figure 1.3). This model is consistent with the "*thrifty-gene*" hypothesis, which states that genes that favor energy conservation in the wild, food-scarce environment, impart genetic risk for obesity and diabetes in the modern, food-rich environment. Maladaptation between an ancestral allele and modern environment arises because natural selection cannot keep pace with rapid changes in the environment. This is particularly true for human environments, which could change completely within a few generations. However, if after the change, the environment reaches a steady state and if the gene has a survival advantage, the ancestral allele changes to a derived allele under positive selection. The derived allele, once again, confers protection and survival advantage, but only as long as the environment does not change. If the environment changes again, the derived allele may or not may not be beneficial. Because many aspects of human environment are continuously changing, there may be a constant mismatch between environment and gene adaptation. This mismatch could provide a selective pressure for genetic adaptation, but could also account for the temporary persistence of several disease-susceptibility genes in the current environment (*the mismatch hypothesis*).

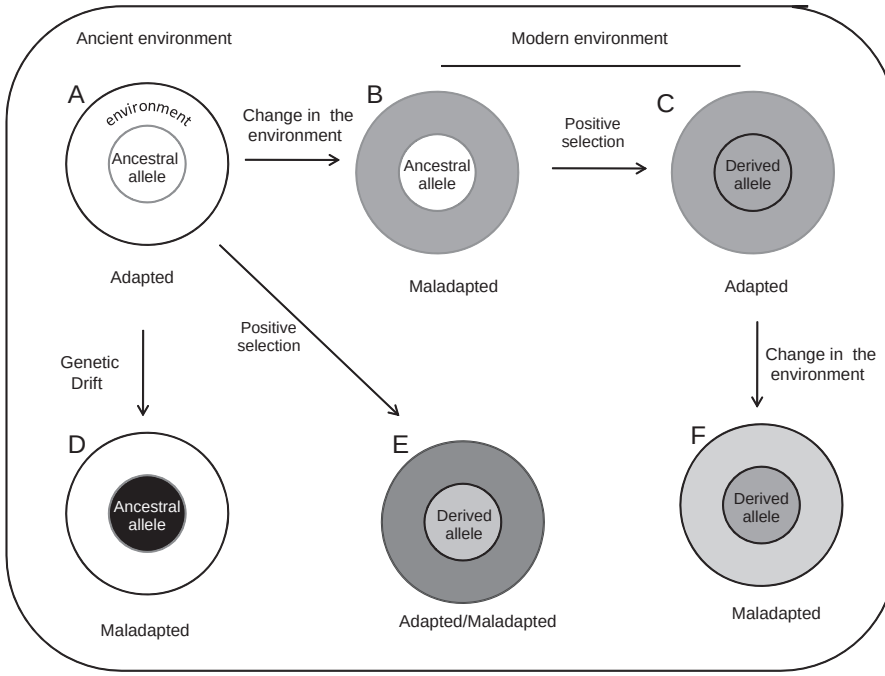

Figure 1.3 Role of environmental changes in disease susceptibility. Ancestral alleles that are adaptive in an ancient environment can become maladaptive in a new environment and thereby increase disease susceptibility. Under positive selection, the ancestral allele may give rise of a derived allele, which is better suited, or more adaptive in the modern environment. The susceptibility of several modern diseases may be high due to a mismatch between the ancestral alleles and the current environment. Adapted from Ding and Kullo.[21]

Another explanation is that the genes that affect CVD susceptibility are retained just by chance (*Neutrality hypothesis*). Because heart disease develops after the reproductive years, it is believed that there are no selective pressures either for or against the genes that regulate CVD susceptibility. When mismatched with the environment these gene variants are not eliminated by a strong purifying selection because they do not impair reproductive fitness. As a result, harmful ancestral genes are retained and tolerated because there is no evolutionary pressure to change them. In addition, new gene variants could arise by genetic drift and these new variants are either beneficial or harmful, but regardless of their effects, these rare variants accumulate because they do not impair reproductive fitness and are therefore not eliminated by strong purifying selection (*the rare-variant-common disease hypothesis*).

A third explanation is that gene variants that drive human evolution by promoting early life survival impair human health in old age. It is believed that one reason that these variants appear and are retained is because they increase reproductive success. Therefore, there is strong positive selection that enriches a derived allele in a population. The derived allele confers a well-adaptive

phenotype and promotes reproductive success; however, this victory comes at a high cost because the very gene variants that were advantageous in youth increase disease susceptibility in old age. Thus, early life acclimatization is optimized at the cost of late-life adaptation so that the derived allele is adaptive in youth but not in old age (since this is victory gained at too great a cost, we can call this the *pyrrhic hypothesis)*. However, regardless of mechanisms, it is evident that the environment plays a leading role in driving the change and in providing context to the derived or retained alleles because it is only within the framework of the environment that a gene is either adapted or maladaptive.

1.1.3 *Out of Africa*

There are several notable examples of how a change in the environment affects the genes that affect CVD susceptibility and how alleles adapted to one environment elevate CVD risk in another environment (Figure 1.3; A to B). A particular interesting example, supporting the mismatch hypothesis is apoE. The apoE gene is the code for a plasma protein that is associated with chlylomicrons and intermediate density lipoproteins (IDLs). ApoE binds to LDL receptors on the liver and is required for the breakdown of triglyceride-rich lipoprotein constituents. Although several polymorphic forms of apoE have been recognized, the most common alleles are ε2, ε3, and ε4. The proteins coded by these genes differ in their affinity for lipoprotein particles and hepatic receptors.[22] The ε4 allele is the ancestral gene present in chimpanzees and other nonhuman primates[23] but it apparently does not impart excessive CVD risk in them (*vide supra*). In humans, however, the ε4 allele is associated with lower apoE levels, higher cholesterol levels and a higher risk of developing coronary artery disease as well as Alzheimer disease (at least in European or Asian populations[24,25]). Why the ε4 allele has become maladaptive in humans in their current environment is not clear, but it is likely that maintaining higher levels of cholesterol may have been beneficial. Cholesterol is required for the synthesis of steroid hormones and neural function and therefore maintaining high levels of plasma cholesterol in the ancient diet-restricted environment may have been advantageous, however, it has become deleterious in the current diet-abundant environment.

Environmental influences could also foster adaptation by positive selection (Figure 1.3; B to C). For instance, the most common apoE variant in the current human populations is ε3.[24] This variant is believed not to have arisen due to genetic drift but to a positive selection, some 100, 000 years ago. Because this change occurred prior to population expansion, it may be reflective of adaptation to a change in diet that accompanied the transition from subsistence to an agricultural economy.[26] This may be because carriers of the ε3 allele were more resistant to the infectious diseases that originate from domestic animals or only become endemic in larger communities that live close together (*e.g.*, smallpox and tuberculosis). The ε3 allele is less strongly associated with CAD and Alzheimer's disease risk than ε4 and it has been suggested to promote reproductive success.[27] Thus, genes can rapidly adapt to new environments and

whether in their ancestral form or in their new adaptive form, their overall contribution to disease risk is entirely contingent on environmental influences and subject to environmental modification. In this regard it is interesting to point out that even in present-day humans, the extent to which apoE variations predict cholesterol levels and disease susceptibility varies with environmental factors such as diet and smoking.[28] Hence, environmental changes can render genetic adaptation not only irrelevant or harmful but they can also modify the risk imparted by a specific genotype.

Hypertension is another CVD risk factor associated with mismatch between environment and genetic adaptation. Hypertension results from the inability of multiple compensatory mechanisms involved in the control of blood pressure to maintain pressure within appropriate limits. Because blood pressure is regulated by many interrelated multiorgan control mechanisms, it is considered to be a higher-order emergent function that depends upon, but is not predictable from, the structures and functions of lower levels.[29] The dominating mechanism for systemic regulation of blood pressure is renal-pressure natriuresis, which controls the set point at which the blood pressure is regulated. In addition, blood pressure is also affected by aging and is significantly influenced by both environmental and heritable components. However, the incidence of hypertension varies widely among different geographic and ethnic origins and it has been estimated that 20 to 30% of interindividual variations systolic blood pressure could be attributable to heritable polygenes.[30,31] However, despite the clear genetic component of blood pressure, essential hypertension shows no clear pattern of inheritance.[32]

A leading explanation of the emergence of hypertension in modern humans is the ''sodium hypothesis''. Salt regulation is a key component of blood-pressure homeostasis and therefore, variable sodium sensitivity could explain the prevalence of hypertension in different human populations. The sodium hypothesis states that when humans first appeared in the hot, dry savannah they adapted to this environment by conserving salt. This adaptation increased their chances of survival in environments where salt was scarce and limiting water loss was advantageous to avoid dehydration. But as humans migrated to more temperate climates, this adaptation became less important. As a result sodium-conserving alleles show strong latitudinal gradients in allele frequency and are more common in Africans than in North Europeans. Many North Europeans retain the sodium-conserving alleles, although others, under positive selection, driven perhaps in large part by climate, have acquired a derived allele, the G(-6)M235 variant in the promoter region of the angiotensinogen gene. In addition to angiotensinogen other genes such as CYP3A5, GNB3, ADRB2 (β2-adrenergic receptor) and SCCN1A also show allelic variation with climate and latitude.[21] The ancestral variants are more prevalent in African populations living near the equator than in those populations that live in northern latitudes. Consequently, rates of hypertension and sodium sensitivity are higher in individuals that carry the ancestral allele in the modern environment (*mismatch hypothesis*) and, therefore, individuals from hot arid climates are more susceptible to hypertension than populations from cold climates.[29] This is borne out by the observation

that African-Americans are at a greater risk of hypertension than Americans of European descent.[33] In contrast, in migrant populations that have moved out of Africa a long time ago, acquisition of a derived allele mitigates the harmful effects of the maladapted phenotype (Figure 1.3; B to C). Thus, changes in the environment render an adaptive allele maladaptive if the components of the new environment interact unfavorably with the ancestral trait (Figure 1.3; A to B). Precisely how this happens in not well understood, however, it is clear that whether an allelic variant of a gene results in an adaptive or a maladaptive phenotype depends entirely upon the environment. Hence, the outcome of a specific genetic makeup is nondeterministic because without relevant environmental triggers the presence of a specific genotype does not necessarily or inevitably cause CVD. A similar predominant role of the environment in modulating the outcome of genetic adaptation could be demonstrated for other processes that contribute to cardiovascular disease such as blood coagulation, inflammation, diabetes and obesity.[21]

These examples demonstrate that the genes (apoE, angiotensinogen, PPARG) that regulate the major CVD risk factors such as hypertension, hypercholesterolemia, diabetes change with the environment. While initially maladapted due to a time lag between the change in environment and natural selection, these traits are under positive selection and there is evidence to support the notion that the derived alleles are more compatible or adapted to the new environment. This is not what would be expected if the major variants of CVD susceptibility genes arose by genetic drift and were not subjected to natural selection or purification. If CVD appears late in life and does not affect reproductive success, why are CVD susceptibility genes under positive selection?

One answer is that genes that regulate the major CVD risk factors such as cholesterol, hypertension and coagulation have pleiotropic effects on the general well being of the individual and are therefore likely to be under significant selection pressure. Because the genes involved in cardiovascular function and health affect the general well-being of an individual, any dysfunctional or maladapted variant is removed readily by negative selection or purification and the derived allele increases survival and reproductive success by providing better cardiovascular adaptation to the environment. For instance, as pointed out earlier, the derived ε3 allele of apoE, in addition to decreasing CVD risk, could increase reproductive fecundity, adaptation to dietary changes, facilitate recovery from head injury or decrease susceptibility to lipophylic pathogens.[34] Thus, the derived allele is selected because of its positive pleiotropy. Similarly, the acquisition of the derived angiotensinogen allele by migrants moving out of Africa has been found to be associated with strong positive selection[29] although the environmental factors that favored the selection of the derived allele or selected against the ancestral allele are not known. Regardless, the genetic traits that favor cardiovascular health are favored in all environments and appear to be under strong selective pressure, even though their direct contribution to reproductive success is uncertain. In contrast, the contribution of genetic drift leading to the accumulation of chance variants (rare-variant common disease hypothesis) is less clear.

Genome-wide linkage analyses have shown that there are no loci for coronary artery disease risk, however, there are some mappable loci with modest effects.[3] Significantly, most of the genes that show weak associations are involved in innate and adaptive immunity, consistent with the notion that inflammation is a critical component of cardiovascular disease. Because a highly active immune response is critical for survival and therefore for reproductive success, it has been suggested that the frequency of these genes is maintained by natural selection even though they increase disease susceptibility in older age (*pyrrhic hypothesis*). However, as we have seen, genes that lower CVD risk in old age (cholesterol and hypertension) are under positive selective pressure. In addition, their contribution to CVD is significantly modified by the environment both before and after the reproductive years. For instance, by studying historical data from cohorts born before the 20th century in European countries, Crimmins and Finch[35] have found that increasing longevity and declining mortality in the elderly occurred among the same birth cohorts that experienced a reduction in mortality at younger age. This is consistent with earlier observations that when life expectancy increased, the increases in the elderly began many decades after the increases in younger ages,[36] indicating that being healthy and disease free at a young age delays the onset of age-associated disease. Thus, what happens before or during the reproductive years does not appear to be irrelevant to aging. That healthy children and adults make healthy seniors is a well-understood euphemism – and for good reason; cardiovascular disease begins early (by some estimates in the preteen years) and therefore environmental and genetic factors that promote good cardiovascular health during childhood and adulthood are likely to decrease the CVD burden in old age. Thus, improvements in the environment, better nutrition, lower infection *etc.*, that improve the CVD health in youth also improve health in old age.

Crimmins and Finch[35] also found that the decline in old-age mortality in their 19th century European cohort was promoted by the reduced burden of infections and inflammation during childhood. They hypothesized that reduced infections at young ages delayed the development of atherosclerotic and thrombotic conditions by reducing the lifetime inflammatory burden. This is consistent with the current view of atherogenesis that holds that lesion formation begins early in life and that an increase in systemic inflammation, due to repeated infections, could accelerate the rate of atherogenesis either temporarily or permanently. Several studies show that by chronically elevating the levels of inflammation, persistent infections could increase the risk of atherosclerotic disease.[36] Thus, it seems reasonable to assume that decreasing inflammation early in life could decrease CVD progression and severity. If this is true it would suggest that CVD health before and during the reproductive age could not be optimized to the detriment of health at old age. Atherosclerotic disease is a lifelong process, and its total burden is the record of the entire environmental life history of an individual and it is likely that few, if any, mechanisms that are beneficial only during youth are detrimental in old age.

The recognition that the environment plays a predominant role in modulating genetic disposition for CVD has important practical implications. If we

could identify the specific environmental triggers and understand how they interact with specific genetic variants it might be possible to prevent much of the disease by altering the environment or modifying its effects on individual with genotypes. For instance, as pointed out by Willet,[37] although phenylketouria is an entirely genetic disease, it could be completely avoided by eliminating phenylalanine from the diet. So, from another perspective it could be viewed as an entirely environmental disease. This perspective is useful, because it can suggest that simple modifications in the environment could significantly impact the outcome of CVD. Further gains can be made by understanding genetic susceptibilities and gene–environment interactions not only within the context of the current environment but also with the understanding of the evolutionary history of how specific genetic adaptations arose and how they modify CVD risk in the current environment. Historically, medical research has focused on mechanistic or proximate causes of disease, but distal evolutionary causes that determine disease susceptibility (or even normal physiology for that matter) are overlooked. Ideally, a complete explanation must be based on a thorough understanding of both the proximate and distal causes. While much has been learned about the mechanistic, cellular and molecular mechanisms, the importance of environmental influences has been underestimated. This is particularly unfortunate because the environment strongly influences both the proximal and distal causes of disease and it is the link and the context within which to understand both the long-term evolutionary causation and the immediate precipitation of disease in a genetically unique individual. In this regard, heart disease (inclusive of metabolic disease such as diabetes and obesity) is a quintessential environmental disease. Its long-term risk is embedded in the evolutionary history of responses to the ancient environment and its current population and individual risks are largely determined by the modern environment.

1.2 Categories of the Human Environment

All life adapts to its environment. For most animals and plants, the environment is primarily the ecosystem populated by natural geographic features and life forms that coinhabit the niche; however, the human environment is more complex. Given this complexity, how can we understand the effects of the environment on humans? What specific constituents or aspects of the human environment influence health and disease? Do they work independently or synergistically? What types of environmental factors are modifiable? Which ones are nonmodifiable? Which parts of the environment affect human health? And are these effects direct or indirect? To address these questions, it is important to understand specific aspects of the human environment and how they affect individual health, disease-risk and mortality. The major difficulty in understanding environmental influences on human health is that the term "environment" is used currently as a catch-all phrase. It is used to describe all physical, social and cultural surroundings of an individual. In this sense it refers to a host

of disparate entities that may be the local climate, food sources or social and economic conditions. Each of these referents, however, has different and unique effects on human health and grouping them together causes these differences to blur. Hence, to delineate the contribution of the human "environment", it is necessary to differentiate among different forms and types of environments.

The term "environment" is derived from the French verb (*en- viron*, circuit) meaning to surround or enclose. If is defined as the set of circumstances or conditions in which a person or community lives, works, and develops. It includes all surroundings, the totality of circumstances and the complex of social and cultural conditions that affect the *nature* of an individual or the society. In its broader sense, it could mean anything that is external to an individual. However, to understand the role of the environment with any degree of specificity we must distinguish between types of environment and its categorically differentiated forms.

From the perspective of an individual, the total environment consists of plastic and aplastic components (Figure 1.4). The aplastic or the nonmodifiable environment is the natural environment that we cannot change significantly. It is the relatively nonmalleable ecosystem that we share will all other living things. This includes the day–night cycle, the season, and our terrestrial rather than aquatic existence – a group of *a priori* conditions that remain relatively constant. There are changes in the natural environment (the seasons, the length of days and nights change), but these are relatively unchangeable by human activity.

In contrast to the natural environment, the *plastic* environment is changeable by human activity. The creation of a plastic environment is the collective work of the community fashioned by its history and culture. The main program of human civilization is to mold the natural environment so as to enhance human safety, comfort, and convenience. However, this self-created, plastic environment completely surrounds and engulfs human lives and it has now become, more so than in the past, the primary domain of our existence. Not only does this environment substitute for our natural ecological environment, it also shields us from nature and it radically modifies our interactions with the natural world. Therefore, to evaluate how the environment affects human disease and health, it is essential to understand not only our interactions with the natural environment, but more importantly, how we interact with the plastic, oysterous environment that we have created around us and how this environment affects our well-being.

Components of the current plastic environment (community, cars, buildings, roads, pollution, *etc*.) have a more profound effect on human health than natural forces or ecological threats (floods, infections, *etc*.). Indeed, the plastic environment has radically changed the natural environment and the current ecosystem itself. While in the past, human gene–environment interactions were primarily driven by changes in the natural environment, ecological changes and geological shifts, the challenge now is to adapt to the ever-changing plastic, man-made environment rather than our natural ecosystem. It is becoming increasing clear that the plastic environment has been a significant agent of natural selection in the past and is likely to be a predominant force in setting our future genetic trajectories.

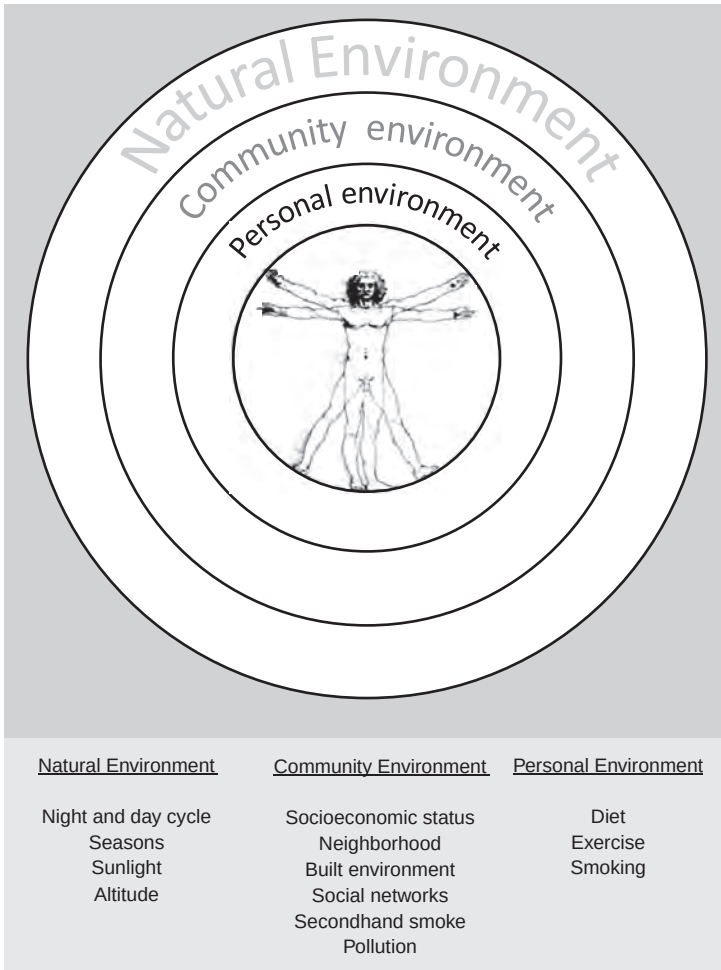

Figure 1.4 Categories of the human environment: Mandala showing the relationship between an individual and the environment. Components of each environment that regulates the risk for cardiovascular disease are listed.

The plastic environment could be further differentiated into a community or societal environment, which is the environment we create as a community and a personal environment that is made up of our own conscious (life-style) and unconscious choices. The community environment includes the social and cultural structures that each generation (like its genes) inherits from their predecessors and contributes to in return. It is the result of the choices that we as a community have made and continue to make. It includes the built environment in which we live (houses, roads, parks) and the environmental conditions (access to healthy food, clean drinking water, *etc.*) that the community (neighborhood, city, country) provides. Recent research has shown that components of

the community and built environment have a significant impact on human health and disease, particularly on chronic illnesses such as heart disease and diabetes. Other physical aspects of the community environment such as noise, pollution, food availability, sanitation, *etc*., also have important health consequences.

The final component of the environment is the personal environment. It is a subset of the plastic environment, but it is not entirely communal. It is populated by the choices that we as individuals make, such as where we live, what we eat, and how we spend our leisure time. These life-style choices have the largest and perhaps the most significant effect on our health. Even though the personal environment is largely created by our own unique choices, these choices are to some extent determined and limited by the community environment. We can only chose from the set of options provided to us by our community. Our freedom to choose is constrained by our community (*e.g*., peer pressure, fashion, advertisement). Still other choices that we make are unconscious because we lack the information to make the right choices or our choices are forced upon us by the community as a matter of tradition, civic laws, *etc*. Regardless, the personal environment is the most malleable, (because it could be changed by one person) and central to our understanding of the effects of other environments (the natural environment and the community environment) and how they are transmitted to a unique individual.

Despite a clear demarcation between the different environmental categories, their boundaries overlap. Human activity has significantly changed the natural environment and it continues to do so. Although, the effects on human activities such as deforestation, pollution of air and water, deep-sea fishing, *etc*., are abundantly evident, there is vigorous debate over the extent to which human activity has changed other major aspects of the natural environment such as global climate or weather patterns. In addition, the boundaries between the communal and the personal environment are continually shifting and in most modern societies there are persistent and opposing attempts to enlarge the influence of one over the other.

1.3 Cardiovascular Disease and the Natural Environment

Recent research provides important insights into how each of the categorically differentiated aspects of the environment affects cardiovascular health. The most ancient of these is our natural environment. In common with all other plants and animals, early human adaptation was in response to the natural environment and the ecosystems in which humans evolved. As we have seen, several genes that regulate cardiovascular function, for instance, the angiotensinogen or the apoE gene variants were selected by the conditions in the African savanna. When humans migrated to different part of the world, different variants of these genes appeared in response to positive selection or random genetic drift. However, as human societies became civilized, the direct impact of the natural ecosystem on human health diminished and natural selection favored adaptation to the new urban environment (*e.g*., retention of the lactase gene;

acquisition of ε4 variant of apoE). It is likely that in the future, the majority of our genetic variation will come from responses to the artificial, plastic environment created by urbanization. Indeed, we are already beginning to see profound effects of urbanization on human health. Nevertheless, the effects of the natural environment cannot be overlooked. We still carry the genes that are adapted to our ancient ecosystem and a disruption of the synchrony between our genes and environment due to differences between the ancient and modern environments is a significant cause of cardiovascular dysfunction and disease.

1.3.1 *Cycles of Night and Day*

An important aspect of the natural environment that affects cardiovascular function is the day–night cycle. The day–night cycle is a fundamental, aplastic feature of the natural environment. All life is entrained to this cycle, which in turn exerts a pervasive control over both plant and animal physiology. Most cells have circadian clock genes that maintain an endogenous 24-h cycle. In the presence of environmental cues (zeitgebers) the master clock, located in mammals in the pacemaker neurons of the suprachiasmatic nucleus (SCN), is entrained to a diurnal cycle.[38] In humans, the SCN sets the intrinsic 24-h cycle accurately to an average of 24 h and 11 min.[39] Light is the main zeitgeber, it regulates the master clock that synchronizes the light-insensitive peripheral clocks to coordinate a 24-h cycle of waking, sleeping, feeding, *etc*. Before the discovery of artificial light, human lives, like the lives of other animals, were synchronized to the cycles of night and day. Extensive research shows that this rhythmic cycle is essential for normal physiology, health, organ growth and tissue renewal and that disruption of this cycle by the plastic environment is a significant CVD risk factor.

Cardiovascular function, as reflected by heart rate and blood pressure, changes rhythmically in synchrony with the day–night cycle. It is lowest at night and during sleep and it begins to rise before waking up, coinciding with a period of vagal dominance, in anticipation of daytime activities. Cardiovascular genes and proteins undergo similar rhythmic changes. It has been estimated that 13% of cardiac genes are under the rhythmic control of the 24-h diurnal cycle.[40] Diurnal variations in gene cycling have also been reported in vascular tissues such as the aorta.[41] Moreover, the intrinsic clock genes are regulated by changes in cell redox, particularly the changes in NAD^+ levels[42] that accompany fluctuations in cell metabolism Changes in NAD^+ levels are significant, because in addition to regulating energy metabolism NAD^+ is also an essential cofactor for the deacetylase Sirt1 (the molecular target of the lifespan enhancing ingredient of red wine – resveratrol[43]). Although the role of Sirt1 in circadian rhythms is not clear, genome-wide acetylation exhibits time-of-day oscillation.[44] Given the recent findings that a large number of the enzymes involved in glycolysis and the TCA cycle undergo acetylation,[45,46] it is likely that diurnal variations in metabolism may be linked to cycles of protein acetylation–deacteylation reactions.

Metabolic processes such as cell growth and tissue repair also oscillate in phase with the day–night cycle. Myocardial proteins are synthesized at the highest rate late in the sleeping period and cardiovascular growth and renewal occurs during the sleeping hours. In addition, neurohormones that regulate cardiovascular function, such as angiotensin II, rennin, aldosterone, growth hormone and atrial naturetic peptide show diurnal variations.[38] Interestingly, it has been shown that rat hearts isolated during their subjective day (dark phase) contract better than those isolated during their subjective night,[47] indicating that the time of the day may be an important regulator of cardiac performance.

In agreement with diurnal variations in cardiovascular metabolism, function and regulation, the incidence of adverse cardiovascular events varies with the time of the day. Myocardial infarctions are most frequent between 6 AM to 12 PM, with most occurring between 3 to 6 AM.[48,49] These events are three times more likely to occur early in the morning than late at night. The frequency of strokes, arrhythmias, abdominal aortic aneurism rupture and sudden cardiac death also shows matutinal clustering between 8 to 11 AM.[50–52] The timing of the onset of adverse cardiovascular events has been linked directly to the intrinsic clock mechanism and does not appear to be related to the stress of waking up. When in a new geographic location, the frequency of cardiovascular events in travelers peaks, for a few days, at times that correspond to their time zone of origin.[38]

In view of the tight rhythmic control of cardiovascular function by the day–night cycle and the clock genes, it is not surprising that disruption of this synchrony has devastating effects on cardiovascular health and that a failure to harmonize internal and external rhythms increases CVD risk. A reflection of this failure is the reported increase in cardiovascular morbidity and mortality in shift workers who are subjected to frequent disturbances in their sleep–wake cycle. Many studies show that shift workers,[53,54] transmeridian flight crews, patients with sleep apnea and other sleep disturbances[38] have higher rates of diabetes, obesity and adverse cardiovascular events. Also, a modest increase in the risk of stroke in women after extended periods of rotating night-shift work has been recently reported.[55] Data showing greater mortality in mice subjected to phase advances of the light–dark cycle, simulating chronic jet lag,[56] provide further support to the view that a mismatch between external and internal rhythms adversely affects health and longevity. In humans, short-term circadian misalignment, similar to that which occurs with jet lag or shift work, results in an increase in postprandial levels of blood glucose and insulin and the mean arterial pressure with a systemic decrease in leptin.[57] These changes may be responsible for the increase in the risk of obesity and diabetes,[58,59] and hypertension[60] in shift workers.

In addition to increasing CVD susceptibility, disruption of the day–night cycle also exacerbates cardiovascular disease. Myocardial infarcts that occur in the middle of the night are larger[61] and angioplasties performed at night are less successful.[62] Animal studies show that the day–night rhythm disturbance increases pressure overload-induced myocardial dysfunction.[38] Similarly, mice subjected to myocardial ischemia-reperfusion at the sleep-to-wake transition

exhibit a dramatic increase in infarct size compared with those at the wake-to-sleep transition,[63] indicating that an inappropriately synchronized wake–sleep schedule may be an important environmental determinant modulating CVD severity. Collectively, these findings suggest "that maintaining normal diurnal body physiology, treating underlying sleep disorders, and/or restoring the endogenous neuroendocrine hormonal profiles, perhaps by imposing a fixed or regular schedule of zeitgebers such as light/dark, rest/activity, or the timing or meals, may significantly benefit cardiovascular health".[38] Thus, recent work in chronobiology reveals an intricate link between a central feature of our environment and cardiovascular function and disease. It shows us that we are inextricably linked to our natural environment and exquisitely attenuated to its primordial rhythms. The synchrony between our endogenous circadian rhythms and the exogenous day–night cycle is of fundamental importance for the normal cardiovascular growth and function, and even seemingly minor disruptions of this primary link have devastating effects on cardiovascular health.

1.3.2 *Four Seasons*

An additional invariant feature of the natural environment is the constant changing of seasons. In most geographical locations there are wide variations in temperature and humidity. The changing of seasons also brings with it changes in the length of day. These changes alter human activity, feeding behavior and the duration of exposure to sunlight. As a result, there are profound variations in cardiovascular health and disease susceptibility. By modifying physiological responses and basic metabolism, seasonal variations affect the expression of CVD phenotype and recovery from adverse cardiovascular events. Although the underlying mechanisms remain mostly unknown, a large number of studies demonstrate that cardiovascular risk factors as well as adverse cardiovascular events show pronounced seasonal variations.

Cardiovascular risk factors – hypercholesterolemia, hypertension, thrombosis, and insulin resistance – show consistent seasonal variations. Cyclical seasonal variations in the circulating levels of cholesterol have been known for the last 80 years.[64] Most studies show that cholesterol levels are higher in winter than in summer. Statistically significant sinusoidal seasonal cycles have been observed in many geographic locations, independent of age, gender, ethnicity, and baseline lipid levels. In general, 3–5% increase has been reported in total cholesterol[65] as well as LDL cholesterol.[66,67] Some studies report that HDL cholesterol follows an inverse pattern; with a peak value in late summer and the lowest value in late winter. On average, the overall difference is 16%,[66] although a decrease in HDL levels in summer has been reported also.[67,68] Nevertheless, plasma low- and high-density lipoprotein cholesterol levels, analyzed similarly, showed synchronous seasonal cycles.[67] In addition, more patients with acute coronary syndromes on statin therapy achieve their target cholesterol level in summer than in winter,[69] indicating that cholesterol synthesis varies with season or that the efficacy of drug treatment is under

seasonal control. Mechanisms underlying seasonal cholesterol cycles remain obscure, although they appear to be relatively independent of changes in ambient temperature, diet or physical activity.[67,70]

Circulating levels of fibrinogen also display cyclical seasonal variation. Like cholesterol, the fibrinogen levels appear to be the highest during winter,[71–73] although some studies have reported peaks in summer[74] or no association at all.[75] Seasonal variations have also been observed in the plasma levels of tissue plasminogen activator antigen and von Willebrand factor.[76] The average seasonal change is between winter and summer months is 10 to 30% or 0.13 to 0.32 g/L.[64] Some investigators have attributed the increase in fibrinogen to concurrent upper respiratory infection especially in old individuals.[72] Fibrinogen is an acute phase protein, which increases with infection. In agreement with this view, a strong association between fibrinogen and other markers of inflammation was observed.[72] However, changes in fibrinogen have also been observed in younger cohorts[66,76] without signs of concurrent infection[66] and therefore do not appear to be always associated with an acute phase response. Such seasonal variations in components of the blood coagulation pathways indicate that the likelihood of adverse cardiovascular events would be higher in winter than in summer months. Indeed, several studies on the seasonal variation in cardiac events show that the frequency of cardiovascular mortality is much higher in winter than in summer.

A significant increase in cardiovascular mortality in winter has been reported by several investigators from all geographic locations both north[77,78] and south[79,80] of the equator. Most of the excessive deaths in the winter months are due to ischemic heart disease although a marked increase in heart-failure deaths has also been reported.[80,81] The difference between the winter peak and summer trough is large. It has been estimated that in England and Wales the winter peak accounts for 20 000 additional deaths per year.[82] Analysis of the 259 000 cases of acute myocardial infarction in 1474 hospitals across the US showed that 53% more cases are reported in winter than in summer.[83] In the entire year, the month of January was the most lethal. In the Australian MONICA study,[84] both fatal and nonfatal coronary events were 20–40% higher in winter than in summer and a 17% seasonal variation was observed in the German Dessau Registry.[85] Data from Los Angeles show 33% more deaths occur in December–January than June through September.[86] These studies suggest that there is a large increase in CVD deaths during the winter months and that this increase could not be attributed to a higher case fatality rate, but that it reflects an authentic increase in the incidence of acute myocardial infarctions. A similar rhythmic seasonal pattern, with a peak in winter, has also been observed for cases of nontraumatic rupture and dissection of aortic aneurysms[87] and stroke.[88]

Several factors can account for excessive CVD mortality in winter. It may be that much of this mortality could be ascribed to susceptible elderly patients with pre-existing disease. Some studies have reported that the elderly are more susceptible to increased winter mortality.[88] This may be because during winter months they are vulnerable to respiratory infections, which trigger an acute phase response leading to exacerbation of cardiovascular disease. However,

excessive mortality has been observed in all ages (<55 to 74 years) at levels comparable to the aged (>75 years),[83] suggesting that the aged are not more vulnerable to excessive winter mortality than the young. Infections, changes in activity levels or diet, however, can only account for part of the excessive mortality associated with winter months, indicating that there may be other explanations for the seasonal pattern of CVD mortality. Although no hard data are available to support any one mechanism, it has been speculated that hemodynamic effects of cold exposure (an increase in sympathetic activity, blood pressure, arterial spasms) could destabilize a vulnerable lesion leading to plaque rupture and occlusive thrombosis.

Cold temperatures, independent of the season, could be an important contributing factor because an excessive number of infarctions has been observed on colder days both in winter and in summer.[89] Exposure to cold temperatures increases vascular resistance and blood pressure, leading to an increase in oxygen demand.[90,91] Cooling of the body is associated with activation of the sympathetic nervous system leading to peripheral vasoconstriction and a decrease in blood flow at rest: a 1 °C decrease in room temperature is associated with a 1.3 mm Hg increase in systolic pressure and 0.6 mm Hg increase in diastolic blood pressure.[92] In the Framingham Offspring Cohort, ambient temperature was found to be a strong determinant of microvascular vasodilation function as measured by hyperemic flow.[93] Low temperature can also increase coronary artery resistance and in some cases induce coronary vasospasm. Changes in temperature can also affect hematologic properties such as blood viscosity and coagulation and even mild surface cooling increases the hematocrit and platelet counts, thereby increasing the likelihood of spontaneous thrombosis.[94] Nevertheless, cold temperatures may not be the only important factor. In some studies, particularly those from the Southern United States suggest that there is an increase in cardiac deaths in summer,[95] and excessive mortality during winter months has been reported even in areas where the temperature is mild throughout the year (*e.g.*, Los Angeles[86]). These studies suggest that changes in the season have a more pervasive effect on cardiovascular function independent of the effects of a change in temperature.

Plasma lipids and fibrinogen levels show seasonal variability and flow-mediated dilation of the brachial artery is the lowest in winter.[93] Together, or by themselves, these changes can trigger plaque rupture. However, the observation that heart-failure deaths are also increased in winter suggests that in addition to plaque rupture, increases in arrhythmia susceptibility, blood pressure or changes in myocardial metabolism *per se* may be important contributors to seasonal clustering of CVD mortality. Direct seasonal variation in cardiac physiology is consistent with the work of Scherlag and coworkers who report that the incidence of sudden cardiac deaths from arrhythmias in dogs subjected to coronary ligation was much higher between November and February than between July and August (42% versus 6%).[96] While these data provide clearer evidence for seasonal variation than the human data (which might be affected by other, social confounders), it remains unclear whether the excessive sudden deaths were due an increase in the sympathetic tone or due to

seasonal changes in myocardial metabolism and excitability. Animal studies also show seasonal variations in cholesterol levels. For example, European badgers, under experimental conditions in which diet was held constant and seasonal weight gains were minimal, show large spontaneous changes in blood cholesterol levels.[97] Whether other animals show similar variations in the levels of cholesterol and other plasma constituents remains unknown, but it is tempting to speculate that because cholesterol is needed to repair injured tissue, and lipoproteins decrease endotoxin injury, the seasonal increase in cholesterol may be an evolutionary adaptation in anticipation of an increase in microbial infections.

1.3.3 *I'll Follow the Sun*

While mechanisms underlying seasonal clustering of CVD mortality remain unclear, a particularly attractive hypothesis is that the increase in cardiovascular mortality during winter may be due to low vitamin D levels. The major source of vitamin D for humans is exposure to sunlight. Diet accounts for a small percentage because only few natural foods contain vitamin D. The photosynthesis of vitamin D involves the conversion of 7 dehydrocholesterol in the epidermis by solar UVB (290–315 nm) radiation to previtamin D3, which then undergoes thermal isomerization to vitamin D3.[98] Vitamin D3 formed in the skin appears in the circulation and it is then transported to the liver where it is converted to 25(OH)D3 – the major index of total vitamin D3 stores. In kidney, 25(OH) D3 undergoes additional hydroxylation to form the biologically active 1,25(OH)2D. Excessive sunlight exposure cannot cause vitamin D toxicity because UVB converts excess vitamin D3 to biologically inert isomers.[99] The efficiency of vitamin D synthesis depends upon the number of photons that penetrate the endothelium. Melanin pigmentation of the skin retards UVB penetration and therefore it decreases sunlight-induced vitamin D synthesis.[100] When exposed to the same amount of sunlight, 20–30% of UVB radiation is transmitted through the epidermis of white skin, whereas in heavily pigmented skin the penetration is $< 5\%$. As a results, individuals with darker skin require a much longer time to synthesize the same amount of vitamin D than those with white skin.[101]

The efficiency of vitamin D synthesis depends upon the extent of exposure to UVB radiation. UVB radiation reaching the earth's surface changes with changing zenith angles. When the sun is low in the sky (during winter or during early morning and late evening) incoming radiation has to travel longer and is subject to more scattering and absorption than when the sun is directly overhead. Consequently, the ability of synthesize vitamin D is affected by the time of the day, the season and the latitude. In northern latitudes (*e.g.*, Boston, 42°N), the filtering effect due to an increase in the zenith angle of sun in winter is sufficient to completely prevent vitamin D3 synthesis from November to February and in Edmonton (10° north of Boston) no vitamin D3 could be synthesized from October to April.[102] Thus, residents in northern latitudes are

likely to face severe vitamin D deficiency in winter months. Indeed, it has been suggested that skin pigmentation in Northern Europeans was lost due to negative selection upon migration from Africa.[103] Hominids in the tropics were probably deeply pigmented; however, as they moved further north, the more deeply pigmented infants were less likely to survive due to bone malformation caused by vitamin D deficiency. As a result, the northern population lost pigmentation due to natural selection. That skin color is an adaptation to maximize UV penetration in northern latitudes (and minimize UV damage in south) is supported by a significant correlation between skin pigmentation and equatorial latitudes in human populations.[103] Despite this adaptation, residents of northern latitudes face constant vitamin D deficiency. Autopsy studies on 19th century residents of Boston, Leiden and The Netherlands show that there was 80–90% prevalence of rickets in children residing in these areas.[104] Even today, living at higher latitudes and being prone to vitamin D deficiency is associated with an increase in the risk of colon, prostate, breast and ovarian cancer, as well as an increased risk of multiple sclerosis, Crohn disease, type-1 diabetes and hypertension.[105] Remarkably, it has been reported that living above 35° latitude for the first 10 years of life was sufficient to imprint on a child a 100% increase in the risk of developing multiple sclerosis independent of where they lived in later life.[104]

Although residents in northern latitudes are particularly prone to vitamin D deficiency, those living in the south are susceptible as well. A seasonal decrease of vitamin D in winter has been reported both in the Northern and the Southern United States. It has been found that that there is 40% greater prevalence of vitamin D deficiency in fall and winter than in summer and spring and that the deficiency was higher in obese children.[106] In the NHANES III cohort, vitamin D deficiency was fairly frequent in younger individuals, especially in the winter/lower latitude subsample.[107] The serum 25-OHD levels in African-Americans were lower than whites, consistent with the higher efficacy of pigmented skin in preventing UVB absorption. Current estimates indicate that globally, 35–80% of children have vitamin D deficiency.[104] In the US, vitamin D deficiency has been found to be common in all groups of adolescents and adults in the winter/lower latitudes subpopulation[107] and 25(OH)D levels are inversely associated with the winter season.[108]

Seasonal and latitudinal variations in vitamin D levels have been associated with geographic and seasonal variations in blood pressure. With increasing distance from the equator, there is a progressive increase in blood pressure that correlates with a gradual fall in ambient UVB radiation.[109] The prevalence of hypertension shows a similar latitudinal distribution. Moreover, blood pressure is higher in winter,[90,109] when UVB levels are low and decreases in summer with the advent of sunnier days. Although it is not clear whether there is a causal relationship between blood pressure and sunlight, it has been reported that exposure to UVB radiation[110] skin tanning in salons[111] or treatment with high-dose vitamin D2[112] reduces blood pressure. In addition, both experimental and epidemiological studies indicate that vitamin D regulates rennin biosynthesis and blood-pressure homeostasis. Disruption of vitamin D signaling in mice

activates the rennin–agiotensin system and induces hypertension and cardiac hypertrophy and in men with the low levels of vitamin D are associated with a 6-fold higher risk of developing incident hypertension.[113] Because vitamin D regulates calcium homeostasis and the secretion of the parathyroid hormone (PTH), chronic vitamin D deficiency causes secondary hyperparathyroidism, which has been linked to an increase in both blood pressure and myocardial contractility.

In addition to blood pressure, vitamin D regulates other cardiovascular functions as well. All cardiovascular tissues express the vitamin D receptor (VDR).[114] This receptor binds to 1,25(OH)2D and the ligand bound receptor, upon association with retinoic acid x-receptor regulates the expression of nearly 200 genes, such as those involved in rennin production, release of insulin by the pancreases, cytokine production by lymphocytes, and the growth of vascular smooth muscle cells and neonatal cardiomyocytes.[114] Overall, 3% of the human genome is regulated directly or indirectly by the vitamin D endocrine system. In mice, the absence of a functional VDR leads not only to a bone and growth plate phenotype, but also high rennin hypertension, cardiac hypertrophy, and increased thrombogenicity.[115] In humans, vitamin D deficiency is associated with an increased risk of hypertension, myocardial infarction, stroke, heart failure, diabetes and peripheral artery disease.[98,114] A correlation between plasma 25-OHD levels and subsequent adverse coronary events has also been observed in the Framingham Offspring Study, which reported that the CVD events were 53–80% higher in people with low vitamin D levels.[116] That these associations may be causal is supported by a recent meta-analysis of 18 randomized controlled trials consisting of 57 000 individuals, which showed that vitamin D intake ($>$ 500 IU/day) decreases all-cause mortality in part by decreasing cardiovascular deaths.[117]

Vitamin D may be a particularly important regulatory factor in obesity and diabetes. Human pancreatic islet cells are capable of calcitriol production[98] and hypovitaminosis D is considered a risk factor for glucose intolerance.[106] Vitamin D status is positively correlated with insulin sensitivity index and individuals with low vitamin D levels display impaired β-cell function and are at a greater risk of developing insulin resistance, type-1 and type-2 diabetes and metabolic syndrome.[98] Supplementation with vitamin D improves insulin resistance, and in one study vitamin D treatment was as effective as troglitazone or metformin in improving insulin sensitivity.[118] Vitamin D is a fat-soluble vitamin and it is readily sequestered in fat, therefore, the bioavailability of vitamin D is obese people is decreased in comparison with nonobese people[108] and in most studies obesity is negatively correlated with plasma 25(OH)D levels and positively correlated with PTH levels.[106] Thus, the obese are likely to be more susceptible to seasonal and latitudinal changes in vitamin D levels. Similarly, vitamin D deficiency in the US is more prevalent in African-Americans than in whites, which may account in part of the high CVD burden in Southern United States, despite lower latitudes and plenty of sunshine.

In Europe, there is a progressive increase in the rates of heart disease from southern to northern Europe. A similar south-to-north gradient is also evident in

the distribution of the ancestral ε4 haplotype of apoE (see above). The proportion of apoE ε4 carriers rises from 10–15% in the south to 40–50% in the north. The retention of the apoE ε4 allele in Northern Europeans has been suggested to be related to better intestinal absorption of fats and a better absorption of vitamin D in the kidneys.[119] Both apoE and vitamin D binding proteins share a common receptor in the proximal tubules. Hence, carriers of the ε4 allele may have been less likely to develop vitamin D deficiency and better fit for living in northern latitudes where the sunlight contains low levels of UVB radiation.

1.3.4 *In High Places*

Altitude is another dimension of the natural environment that affects cardiovascular health and disease. Nearly 150 million people live in areas that are more than 2500 m (8200 feet) above sea levels.[120] Populations living at high altitude have adapted to cold temperatures and low oxygen levels. There are significant anatomical, physiological, metabolic and biochemical differences in the cardiovascular system and functioning of highlanders and sea-level dwellers. For instance, it has been reported that although right ventricular hypertrophy at birth decreases promptly in newborns at sea levels, it persists throughout life in Andean children living at 4540 m.[120] Moreover, the level of altitude has an inverse relationship to arterial oxygen saturation and a direct relationship to pulmonary artery pressure. As a result, some human and animal populations living at high altitudes have right ventricular hypertrophy and thick pulmonary arteries. Alveolar hypoventilation in susceptible highlanders leads to chronic mountain sickness.[121] It is characterized by excessive erythrocytosis, severe hypoxemia, and pulmonary hypertension, which often evolves to cor pulmonale, leading to congestive heart failure. CMS prevalence varies between 5–8% in various populations of highlanders and it increases in association with lung disease, obesity, smoking and environmental pollution.[121]

Of the several highland populations, native Tibetans and Nepalese sherpas appear to be most well-adapted to living at high altitudes. Tibetans have the oldest altitude ancestry in the world and have through successive generations attained a high grade of adaptation to high altitudes; perhaps by natural selection. The prevalence of chronic mountain sickness in Tibet is low (1.2%).[120] In contrast with sea-level dwellers (Chinese Han immigrants, or Caucasians), Tibetans show lower pulmonary pressure response to exercise with less increases in ventilation rates and better preservation of cardiac output. They have greater ventilatory capacity and hypoxic ventrilatory response as well as greater physical performance. Interestingly, Andean natives who are have a shorter history of living at high altitudes than Tibetans are less well adapted. Autopsies of Andeans frequently show greater muscularlization of the distal pulmonary arterial branches and right ventricular hypertrophy.[122] A similar difference is evident in animals. Species native to mountainous areas (yaks, snow pigs, pika) have better cardiopulmonary responses than domestic animals recently transported to high altitudes.[120]